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MYCOLOGICAL FINDINGS IN SPUTUM*

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VARIOUS fungous forms have been known for generations to play important rôles in the industrial world, particularly in the manufacture of dairy products and beverages. These organisms are responsible for the flavor, good and bad, of many of these products. In the dairy world the bad taste of butter, the foaminess of cream, and the unpleasant flavor of certain cheeses are a few of the ill effects of contamination with the fungi. Grain rust, smut of corn, plant rot, the disease of wheat known as ergot, and other diseases far too many to enumerate here are all due to the organisms belonging to the fungus group. While all this has been known for years in the industrial world, it is only within recent decades that the importance of the fungi has become recognized by medical investigators as the etiologic agent in diseases other than skin disorders and most particularly in the diseases of the respiratory tract. With this in mind sputum specimens were studied to determine how often and what kinds of fungi could be found in the sputum, with and without etiologic relationship.

GENERAL METHODS

Mycological studies were made on all specimens of sputum which were sent to the laboratory. These studies included specimens from 101 patients from the Desert Sanatorium and its outpatient clinic, and from 40 patients from the Southern Pacific Hospital in Tucson. Forty-seven patients from the Desert Sanatorium had a diagnosis of pulmonary tuberculosis. Tubercle bacilli were recovered from the sputum of all of these patients. Fourteen patients had bronchopneumonia, 7 had asthma, 8 had bronchiectasis, 8 had sinusitis,

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8 had severe colds, 2 had carcinomatous involvement of the lungs, 3 had chronic nonspecific pulmonary infections, 1 had an undiagnosed pulmonary infection with probable malignancy, 1 patient had esophageal diverticulum with a possible mediastinal abscess, and 2 patients were undiagnosed until mycotic infections were discovered. The patients from the Southern Pacific Hospital all had pulmonary tuberculosis. In addition to the tuberculous infection one patient had pneumonoconiosis, one had diabetes, and a third had atrophic arthritis.

Individual specimens were examined for the presence of fungi from one to ten times. The total number of sputum examinations were 373. The specimens varied from single expectorations to specimens collected over a period

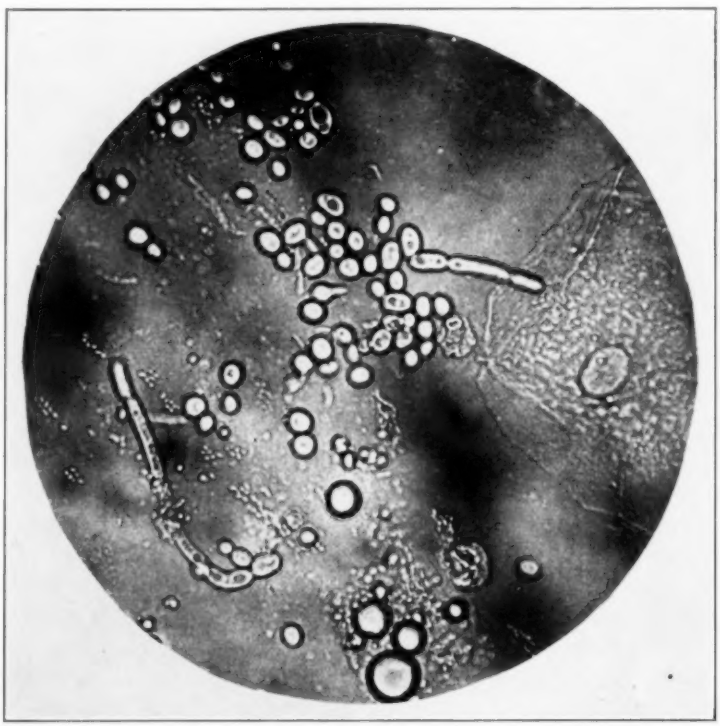

Fig. 1.—Budding form of monilia in fresh sputum, together with fat globules, epithelial cells, and bacteria (Case DrG).

of twenty-four hours. One specimen was a thirty-six-hour collection, the total amount of sputum at that time being 6 gm. The specimens were all collected in closed containers. Each specimen was searched for suspicious particles—the typical small grayish masses analogous to the sulfur granules of actinomycotic infections. These masses contain the mycelium and encysted forms together with fibrin, epithelial cells, and leucocytes. Fig. 1 shows *Monilia albicans* as it appeared in a fresh specimen of sputum (Case DrG). The yeastlike forms and beginning hyphae are seen in the photograph together with epithelial cells, bacteria, fat droplets, and leucocytes. Particles were examined microscopically and cultured directly upon Sabouraud's glucose agar. The presence of these particles, as Stovall and Bubolz[1] have pointed out, tends to associate

them with the exudate and secretions coughed up from the bronchi. Accidental contamination may in this way be ruled out. Accidental contamination may be picked up on culture but rarely on wet preparation.

It was not possible to make a study which included clinical follow-up investigations owing to the nature of the patients seen at the Desert Sanatorium, where admissions are largely seasonal, with many patients coming for the winter months only and remaining in Tucson for a period of from a few weeks to a few months. Some came for diagnostic studies only and were in the clinic for as brief a period as a few days. The available material was utilized, and

TABLE I

CASES WITH DIAGNOSES AND MYCOLOGICAL FINDINGS*

CASE	CLINICAL DIAGNOSIS	FUNGUS ISOLATED FROM SPUTUM	NO. OF SPUTUM EXAMS.	NO. OF POSITIVE EXAMS.
WmS	Pulmonary tuberculosis	*M. albicans* Endomyces	2	2
MH	Asthma	*M. candida*	1	1
ACh	Undiagnosed pul. infection with probable pul. malignancy	*M. albicans*	6	6
GR	Pulmonary tuberculosis	*M. albicans*	3	3
EP	Pulmonary tuberculosis	*M. albicans*	8	8
KSP	Pul. tuberculosis Atrophic arthritis	*M. albicans*	1	1
FrD	Chronic nonspecific pul. infections	*M. albicans*	6	6
DrG	? Pul. tuberculosis ? ? Lymphogranuloma ?	*M. albicans*	5	5
WmSh	Sinusitis	Saccharomyces Torula	1	1
GZ	Pul. tuberculosis	*Coccidioides immitis*	7	3
PT	Coccidioidosis	*Coccidioides immitis*	5	5
HS	Streptococcal pneumonia	*Coccidioides immitis*	1	0
MC	Chronic nonspecific pul. infection	Sporothricum	1	1
CK	Pul. tuberculosis	*Aspergillus fumigatus*	10	10
WmH	Pul. tuberculosis	*Aspergillus fumigatus*	4	3
SWL	Nonspecific pul. infection	*Aspergillus niger*	2	1
RC	Bronchiectasis	*M. albicans*	6	6

*This chart lists examinations performed before any form of antimycotic treatment was administered.

this study is of necessity a clinical laboratory study, and not a combined clinical and laboratory study. The only case in which an autopsy was performed was in the case of HS.

RESULTS

In this series of 141 patients, 17 had positive mycological findings. Aspergilli and coccidioides were each found 3 times. Monilias were found in the sputum of 8 patients; sporothricum, endomyces, torula, and saccharomyces were found in the sputum of 3 patients, 2 patients having double mycological findings. In the sputum of one patient torula and saccharomyces were found, in the other monilia and endomyces. Table I shows the cases with their original

diagnoses, the mycological findings together with the number of times each sputum was examined and found positive. Because it is not possible in this series of cases, with but two exceptions, to determine whether or not the fungous organism was the etiologic factor in the pulmonary infection, the mycological diagnosis as the disease entity is not included in the chart. In most cases the fungi were in all probability secondary invaders, but that question is entirely outside the scope of this report.

ANALYSIS OF ORGANISMS STUDIED

Yeastlike Organisms.—The yeastlike organisms, saccharomyces, torula, endomyces, and monilias are an important group. In view of the reports of Maher and Marett it was surprising to find monilias in so few of the sputa. Maher[2] reported monilias in the sputum from all of his cases of pulmonary

TABLE II

CULTURE	TYPE ACCORDING TO FERMENTATION REACTIONS	DOSE IN MILLIONS PER 100 GRAM RABBIT WT.	RESULT	POSTMORTEM FINDINGS
WmS	*M. albicans*	1.5	Died 48 hr.	Generalized moniliasis
MH	*M. candida*	0.5	Well 6 wk.	No evidence of moniliasis
		2.5	Well 8 wk.	
		6.0	Well 4 mo.	
		25.0	Died 21 days	Generalized moniliasis
ACh	*M. albicans*	0.5	Died 6 days	Generalized moniliasis
GR	*M. albicans*	0.75	Died 6 days	Generalized moniliasis
EP	*M. albicans*	0.5	Died 6 days	Generalized moniliasis
KSP	*M. albicans*	0.5	Well 6 wk.	No evidence moniliasis
		1.5	Died 5 days	Generalized moniliasis
FrD	*M. albicans*	0.5	Well Killed 6 wk.	No evidence moniliasis
		1.5	Killed 9 wk.	Mycelia and cysts in bile
		3.0	Died 36 hr.	Generalized moniliasis
DrG	*M. albicans*	0.66	Died 72 hr.	Generalized moniliasis
RC	*M. albicans*	1.0	Died 96 hr.	Generalized moniliasis

tuberculosis when he used milk as a medium. Marett[3] states that he grew monilias in one hundred per cent of his cases of tuberculosis. In another series of cultures Marett[4] found fifty per cent of all of his sputum specimens to contain monilias.

Seven of the monilias isolated in this series of cases were *M. albicans*, one was *M. candida*. A classification of the monilias was first made according to the classification of Stovall and Pessin[5] by pathogenicity tests for the rabbit by intravenous inoculation of a measured number of organisms. They found that *M. albicans* was pathogenic for the rabbit in doses of from 0.5 to 1.5 million organisms per hundred gram of body weight. *M. candida* requires doses of from 6 to 35 million organisms per centigram of weight. *M. parapsilosis* is not pathogenic for the rabbit in any dosage. The results of the pathogenicity tests of monilias isolated in this survey are shown in Table II. One culture (strain MH) required 25 million organisms per hundred gram of weight to kill the rabbit, thus classifying the organism as *M. candida*. All the other strains were *M. albicans*.

Stovall and Pessin[5] have carefully described the pathologic findings in experimental moniliasis in rabbits by intravenous inoculation. The lesions were distributed throughout the body, involving the skeletal muscle, brain, spleen, lungs, with an intensity of reaction in the kidneys and relatively slight, if any, involvement of the liver, although the bile nearly always shows abundant yeastlike cells and many long mycelia.

The finding of monilias in the bile of the experimental rabbits in this series was fairly constant. Strain FrD which was of low virulence for the rabbit produced no gross lesions when inoculated in relatively small doses. One animal receiving 0.5 million organisms per hundred gram of its weight was sacrificed six weeks later. There were no gross lesions. Monilias were

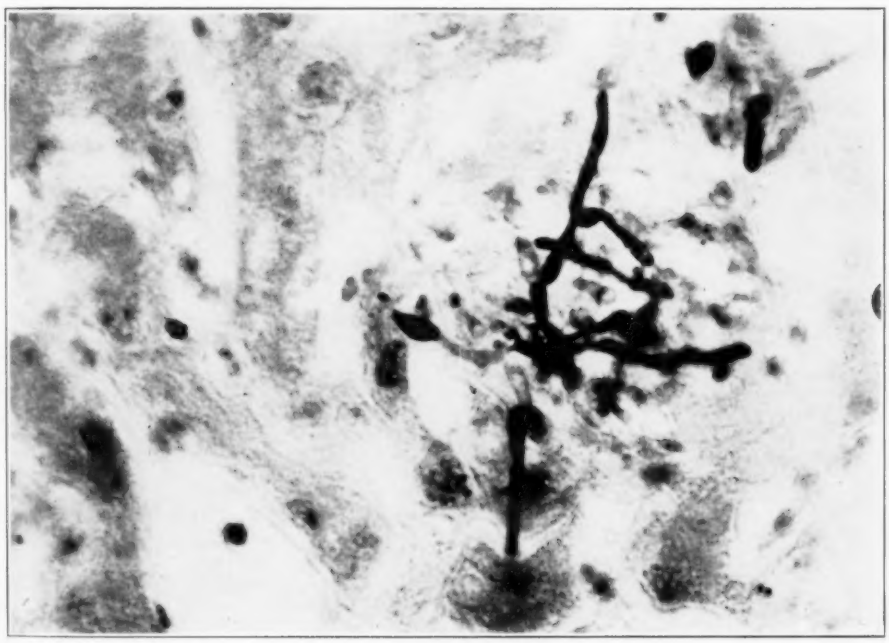

Fig. 2.—*M. albicans* in liver of rabbit (Culture WmS).

not recovered on culture from the kidneys, lungs, brain, or bile. A second rabbit inoculated with 1.5 million organisms per hundred gram rabbit weight was sacrificed nine weeks later. The bile was found to contain, on microscopic examination, a few organisms, both cells and mycelia. This was the sole positive finding in this animal. Cultures of the urine, kidneys, lungs, heart, and brain yielded no monilias. It required 3 million organisms of this strain per hundred gram weight to produce generalized moniliasis and ensuing death of the animal. Table II shows the lethal dosage of the monilias isolated in this study. The only organism at variance with the classification of Stovall and Pessin was this organism from the sputum of FrD.

With the strain WmS death was produced in the rabbit in forty-eight hours by intravenous inoculation of 1.5 million organisms per hundred gram of the rabbit's weight. In this case the liver showed small abscesses contain-

ing a large number of organisms. The entire outer surface of the liver had a necrotic appearance. Fig. 2 shows a section of the liver containing the organism. With the strain KSP the liver of the experimental animal showed considerable enlargement. It weighed 116 gm., which was 6 per cent of the animal's total weight. (The weight of a normal rabbit liver as measured in this laboratory is about 2.5 to 3 per cent of the animal's total weight.) Gross

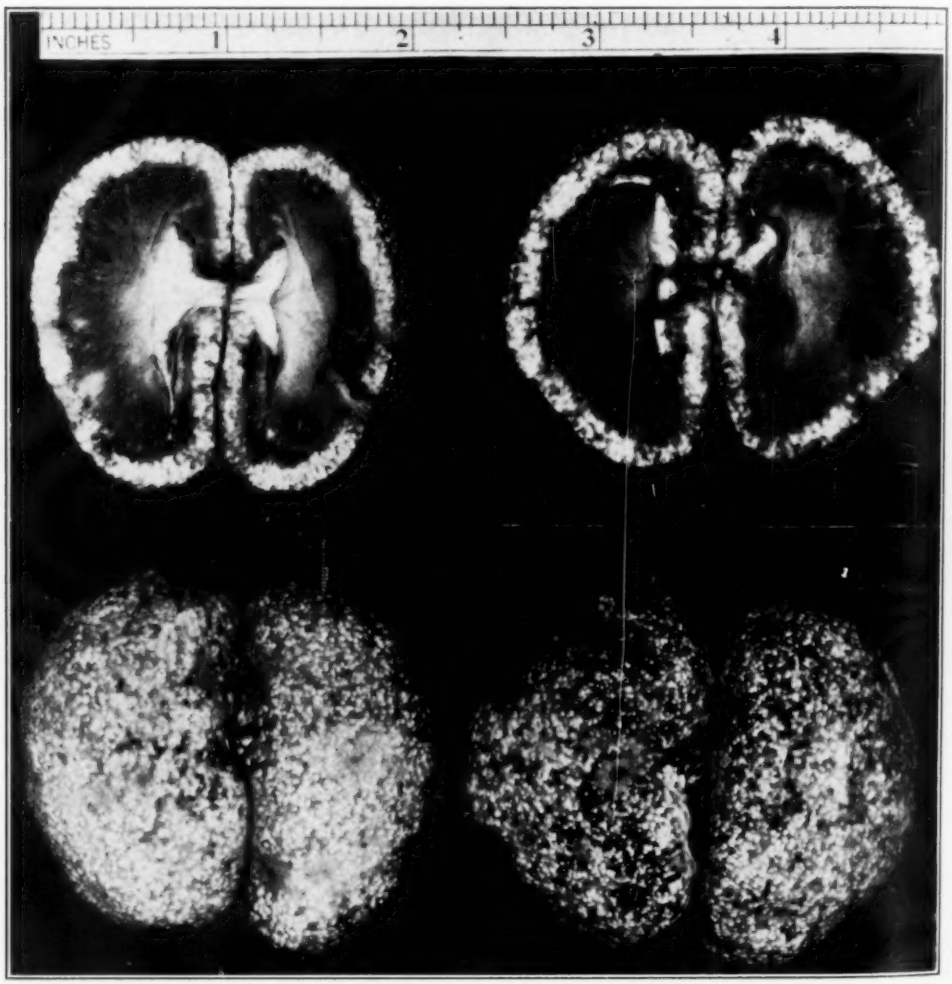

Fig. 3.—Kidneys of rabbit inoculated with *M. albicans* (Culture KSP).

lesions were not numerous in this animal's liver, but by histologic examination many organisms could be found. Fig. 3 shows the kidneys of this same animal. The kidneys containing innumerable small abscesses were enlarged to more than twice the normal size. Fig. 4 in comparison shows the kidneys of an animal inoculated with *M. candida* (strain MH). The pinpoint abscesses are rare. The kidneys are not enlarged.

One of these cases (Case RC) had what appeared to be a primary monilia infection. The patient had the typical severe paroxysmal cough, with large

amounts of tenacious, purulent sputum in which *M. albicans* was repeatedly found. No tubercle bacilli were found on smear by culture or by animal inoculation. He showed toe and finger nail involvement characteristic of this disease. After iodide therapy, his sputum decreased in quantity, changed in character from purulent to mucoid. *M. albicans* could no longer be found on smear nor by culture of concentrated sputum.

The torula organism, another of the yeastlike organisms isolated in this survey, was the red or pink variety characterized by the deep salmon-pink color of the culture. The organism was isolated from the sputum of a young man (Case WmS) with sinusitis. A saccharomyces cerevisiae was isolated from the sputum at the same time. The red torula is apathogenic to rabbits by intraperitoneal and intravenous inoculations, and to mice and guinea pigs by intraperitoneal inoculations of large doses. Henrici[6] has called red torula one of the constant contaminants of the bacteriologic laboratory. This, however, was the only time that the red variety of torula was encountered in this laboratory.

Fig. 4.—Kidney of rabbit inoculated with *M. candida* (Culture MH).

Red torula has been reported elsewhere in sputum.[7] Stovall and Bubolz have also found the "pink" variety in sputum. Taber[8] reports a fatal case of pulmonary infection with the salmon-pink torula in which the organisms were recovered from the lungs at autopsy.

Saccharomyces also may be found in sputum, but little can be found in the literature of this country regarding its possible etiologic rôle. It is sometimes found associated with other yeastlike organisms. It has frequently been found in cultures of the tongue, tonsils, and teeth in this laboratory. Two series of cultures isolated from sputum were sent to this laboratory from other laboratories in which saccharomyces was associated with another yeastlike organism. In each case it was in combination with endomyces.

The endomyces isolated from the sputum of WmS was of low virulence for laboratory animals. It failed to infect mice by intraperitoneal inoculation, guinea pigs by subcutaneous and intraperitoneal inoculation, and rabbits by intravenous inoculation. An intracardiac inoculation into a guinea pig pro-

duced death in two weeks. The lungs showed multiple abscesses from which the organism was recovered. Histologic examination of the lung tissue revealed the yeastlike forms only. The culture was characterized by heavy mycelia, many yeastlike forms, and a few spores. The spores could be demonstrated after thirty-three days' growth on carrot agar.

Sugar fermentation tests were made on all the yeastlike organisms isolated in this study. At the same time, fermentation reactions were determined on stock cultures of *M. albicans*, *M. candida*, *M. parapsilosis*, torula histolytica, endomyces and saccharomyces cerevisiae obtained from Dr. W. D. Stovall, of the University of Wisconsin. At first a wide variation of reactions was produced, not only with the recently isolated strains but with the stock cultures as well. It may have been the age of the culture or the size of the inoculum which was responsible for the variation in reactions as suggested by Hopkins and Hesseltine.[9] They found that both of those factors, together with the personal equation of the reader, caused a variation in final results.

Stovall and Bubolz[1] reported a constancy of fermentation reactions with 250 cultures of monilias from which they reduced the heretofore complex classification to three species. Fineman[10] also classified the monilias of thrush by fermentation tests and reported constant reactions over a long period. Dodge[11] and Dalmau[12] are both of the opinion that sugar reactions are variable, certain strains even losing their power under certain conditions to ferment sugars which once they did ferment. Lamb and Lamb[13] have employed biochemical and serologic methods for differentiating and correlating the various species of monilias. They likewise have placed all the monilias into three groups, although their grouping did not coincide with that of Stovall and Bubolz. They agree, however, that *M. parapsilosis* is a group entity. Reed and Johnstone[14] isolated 31 strains of yeastlike organisms from the stools of 50 patients. Twenty of these were monilias. They classify their monilias into Stovall's types I, II and III and add types IV, V, and VI of their own classification on the basis of sugar fermentation reactions. In their study animal inoculations were not made. Boné[15] combined the two classifications by placing a series of cultures isolated from human sources into groups II and III of Stovall and Bubolz by fermentation reactions, and into group I of Lamb and Lamb by serologic tests. Boné did not isolate any organisms of *M. parapsilosis* group in his study of human material, nor was *M. parapsilosis* isolated in this work with sputum.

When the technique of Stovall and Bubolz[1] was followed in this laboratory, using purified sugars in a 1 per cent solution in broth made of Leibig's extract, bromthymol blue as an indicator, and adjusting the pH to 7.2, results were obtained compatible with those of Stovall and Bubolz, who have greatly simplified the classification of the monilias and other yeastlike organisms. Pfanstiehl's sugars were used throughout the experiment. The growth of a forty-eight-hour culture on Sabouraud's glucose agar slant was suspended in 6 c.c. of sterile distilled water. One-tenth of a cubic centimeter of this suspension was inoculated into each sugar tube and into milk. Milk medium made according to Stovall and Bubolz[1] with the addition of calcium lactate was used for the determination of coagulation. Many series of tests were made employing this

technique. Varying lots of Pfanstiehl's sugars were used, such as old stock sugars which had been in this laboratory and in the bacteriologic laboratory of the University of Southern California Medical School for several years, and freshly purchased sugars from several different chemical houses. With each "run" the results were identical for all practical purposes. Occasionally a strain would show production of a trace of acid in dextrin.

The reactions of the yeastlike organisms in dextrose, levulose, mannose, maltose, galactose, sucrose, lactose, dextrin, inulin, raffinose, and milk were determined to aid in the differentiation of the organisms. It was decided that the reaction in lactose, dextrose, levulose, maltose, sucrose, and milk were sufficient, however, for diagnostic procedures, and the use of the larger number of sugars was discarded. The monilias, Stovall's types II and III, were easily differentiated by sugar reactions and coincided with the classification already made by the animal pathogenicity experiments, as well as with the reaction of the stock cultures of Dr. Stovall.

The endomyces was differentiated from a monilia by sugar fermentation reactions before the formation of ascospores could be demonstrated. *M. candida* and *M. albicans* both produced acid and gas in maltose while the endomyces showed neither acid nor gas production. In this study the red torula was easily classified by its characteristic bright pink color and sticky viscous growth on culture media and morphologic appearance of oval cells, producing neither mycelia nor asci. This particular culture did not produce pseudomycelia. It fermented dextrose, levulose, mannose, and sucrose without the production of gas. Maltose was not fermented. The saccharomyces, on the other hand, fermented dextrose, levulose, mannose, sucrose, and maltose with the production of gas. Lactose was not fermented by any of the yeastlike organisms isolated in this group. The sugar fermentation tests were of definite value in the classification of these organisms isolated from sputum.

Aspergilli.—Of the three aspergilli isolated in this survey two were identified as *Aspergillus fumigatus*, according to Dodge[11] by cultural and morphological characteristics. Aspergilli were found consistently over a period of two years in the sputum of two patients. In three of the four specimens of WmH sent to the laboratory aspergilli were found. Between the second and third specimens, a period of fourteen months had elapsed. The sputum still showed aspergilli but was negative for tubercle bacilli by animal inoculation. Seventeen months later, when the patient had been pronounced clinically cured of tuberculosis for nearly two years, he sent another specimen of sputum to the laboratory for examination for acid-fast bacilli. At that time no aspergilli could be found by wet preparation nor by cultural methods. In this case the aspergilli remained in the sputum longer than the tubercle bacilli.

The sputum of CK was examined many times over a period of two and a half years and found to contain aspergilli on all occasions. The patient had large tuberculous cavities. When first examined here, the sputum contained large numbers of tubercle bacilli, caseous particles, elastic fibers, and numerous aspergilli. Examinations made every month for the next eight months continued to reveal aspergilli in the sputum. The patient left the sanatorium

but returned five months later, at which time aspergilli could still be demonstrated in his sputum. He again left the sanatorium and returned eight months later. *Aspergillus fumigatus* was at that time present in his sputum. No further studies were made. The patient left and did not return.

The aspergillus cultures from the two patients were identical in their biological reactions. Localized abscesses were produced in guinea pigs following subcutaneous inoculations of a relatively small dose. No generalized infections were ever found. Cultures of the abscesses yielded a profuse growth of aspergilli in pure culture. The spores, but no mycelia, could be demonstrated in the lesions. A series of intravenous inoculations into chickens in large doses failed to produce any lesions. These aspergilli were likewise nonpathogenic on repeated intravenous inoculation into pigeons and rabbits. Pigeons fed grain soaked in a suspension of the organisms failed to develop any lesions.

One culture of *Aspergillus niger* was obtained from a patient with chronic nonspecific pulmonary infection of long duration. Only two specimens of sputum could be obtained from this patient. The first time the sputum was examined, the typical black particles were found in the sputum which on microscopic examination showed mycelia and spores. Cultures of these particles yielded *Aspergillus niger*. The second specimen submitted to the laboratory two weeks later showed no suspicious black specks. Cultures were all negative for aspergillus. The patient left Tucson without sending any more specimens to the laboratory. In view of the fact that the black particles containing the spores and mycelia were found in the sputum, it did not seem justifiable to call the cultural findings a laboratory contamination.

The rôle of the aspergilli in these cases was undoubtedly that of a secondary invader. Bethune and Moffatt[16] showed that *Aspergillus niger*, when superimposed on regressing lesions of experimental tuberculosis, showed no power of invading the tissue nor did it produce any mycelia. It remained purely as a saprophyte. Kampmeier and Black[17] reported *Aspergillus niger* in association with bronchial carcinoma, again as a saprophytic organism. However, primary cases of aspergillosis have been reported as far back as 1842 by Bennett and in 1856 by Virchow,[18] and in recent years by Schneider,[19] Jacobson,[20] and Craven,[21] as well as many others.

Coccidioides immitis.—Of the three cases of coccidioides infections one was of particular interest in that the organisms appeared on postmortem cultures from pus from an abscess in the lung. The infection was apparently not suspected during the lifetime of the patient. The patient HS entered the sanatorium acutely ill, and died three days later of pneumonia. He was too ill to be questioned or to give any medical history. However, it was learned from his daughter that he was sixty-seven years old and, while not of robust health, was never considered an invalid. He had been in the habit of spending his winters in Arizona. At autopsy* two types of lesions were found in the lungs, typical bronchopneumonic foci in which no coccidioides cysts could be demonstrated, and numerous small and larger abscesses, many with completely necrotic centers in which a few coccidioides cysts were found. Cultures of

*Autopsy report by Dr. Max Pinner.

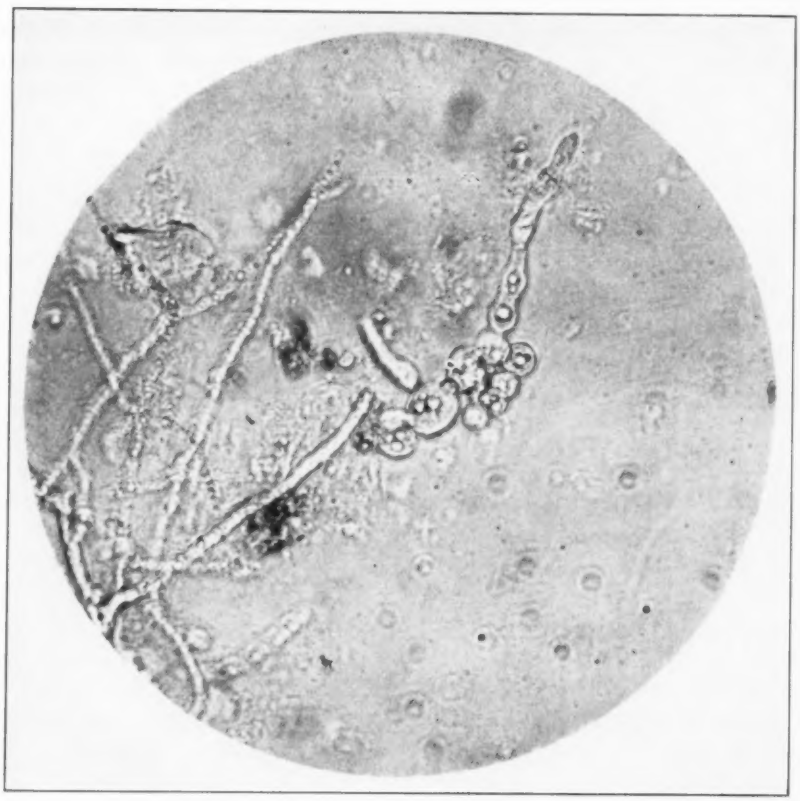

Fig. 5.—Coccidioides in sputum.

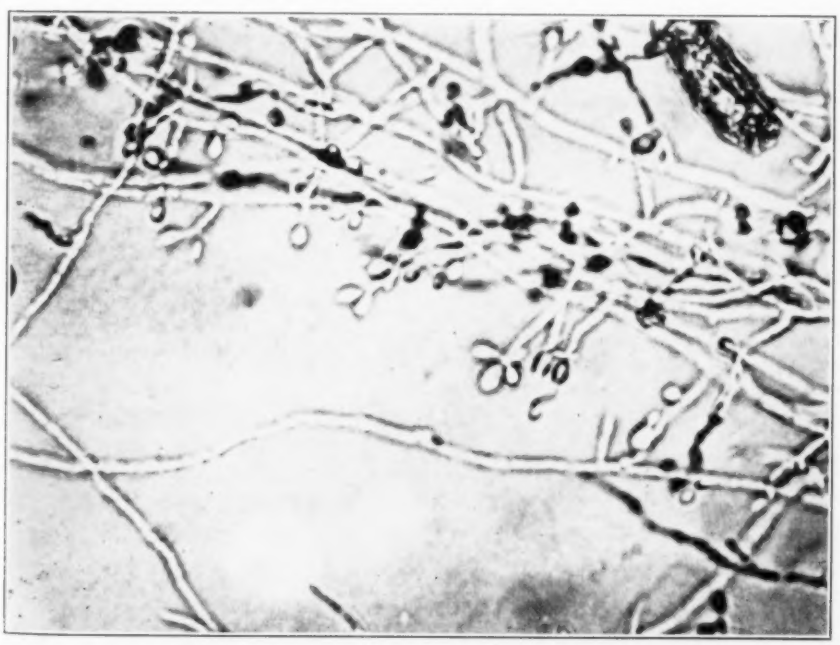

Fig. 6.—Slide culture of sporothricum (Culture MC).

these abscesses yielded *Coccidioides immitis*, pure cultures of which produced typical lesions in guinea pigs, mice, and rabbits when inoculated intraperitoneally.

The second case of coccidiosis (GZ) was the only patient in this series in whom a fungous disease was suspected. The patient came to the sanatorium referred by a physician from an eastern state. The physician had diagnosed his case as pulmonary tuberculosis, complicated by a mycotic infection of unknown origin. The referring physician had noted in the sputum the presence of "granules," which he said contained pus, very few organisms, and no acid-fast bacilli. An "unidentified mold" had been cultured from one of these "granules."

Upon arrival here several weeks later no such "granules" could be demonstrated in the patient's sputum. The sputum was negative for fungi by microscopic and cultural examinations. Acid-fast organisms were present in large numbers. These organisms produced typical inoculation tuberculosis in guinea pigs. The patient seemed better and left the sanatorium, and was placed under the care of the outpatient department.

Five months later a granuloma appeared on his leg. Cultures from this lesion yielded *Coccidioides immitis* in pure culture. His sputum was again examined, this time revealing the typical double contoured cysts of coccidioides.

The third case (PT) of coccidioides was that of a seventeen-year-old boy. He had a slight cough following what he said was a cold, but no other complaints, and said that he felt fine. A roentgenologic examination revealed what appeared to be a rapidly developing pulmonary tuberculous cavity. Repeated examinations revealed no tubercle bacilli. In this case no "granules" were seen. The sputum was concentrated with sodium hydroxide. Coccidioides cysts were found in the concentrate. Fig. 5 shows a group of coccidioides cysts beginning to grow, together with some long mycelial threads, the sputum acting as a culture medium. Cultures on Sabouraud's glucose agar of bits of the sputum picked at random yielded many colonies of *Coccidioides immitis*. Guinea pigs inoculated with the culture as well as with fresh sputum showed typical inoculation coccidioidosis.

Sporothricum.—The sporothricum culture is a little difficult to explain. The organism was isolated from a localized abscess at the site of inoculation of a guinea pig injected with a fresh, undigested specimen of sputum. Fig. 6 shows a culture of the organism with its characteristic stemmed, pear-shaped conidia. The patient (MC), an elderly woman, had complained of respiratory infections over a period of several years. She entered the sanatorium complaining of extreme fatigue, anorexia, and a constant cough which was mostly nonproductive. She remained in the sanatorium for two weeks. Only one specimen of sputum was sent to the laboratory. This specimen was negative for tubercle bacilli both by animal inoculation and by culture. The roentgenologic findings did not rule out a mycotic infection. The patient did not return after this first admittance to the sanatorium. At autopsy of the guinea pig inoculated with the sputum of MC no tuberculous lesions could be found. There was an abscess at the site of inoculation, cultures of which yielded the sporothricum. It is not possible to state definitely whether the sporothricum came from the sputum or was a secondary invader into the

guinea pig tissue. Sporothricum is found with great difficulty in wet preparations and undoubtedly would have been overlooked in the microscopic examination of the wet preparation.

SUMMARY

Mycological examination of the sputum of 141 patients is reported. Seventeen patients harbored fungi in their sputum. Specimens were examined from one to ten times, making a total of 373 specimens examined. Yeastlike organisms were most commonly found. Coccidioides appeared three times. Two patients had double findings, endomyces and *M. albicans*, and saccharomyces and torula. A sporothricum was isolated under peculiar circumstances. Two patients showed the presence of aspergilli in their sputa over a period of two years. Fermentation tests, morphologic structure, and animal pathogenicity methods were used in classifying the various types of fungi isolated.

I wish to acknowledge my indebtedness to Dr. John F. Kessel, of the Medical School of the University of Southern California, who made the completion of this report possible.

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BLOOD COPPER AND IRON IN RELATION TO MENSTRUATION*

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THE effect of menstruation upon the composition of the circulating blood has recently received much attention. Of special interest has been the work of those investigators attempting to correlate hemoglobin and erythrocyte variations with different periods of the menstrual cycle. The findings of different workers are not in agreement. Several investigators including Merletti[1] and Reinl,[2] have found a decrease in the number of erythrocytes in the premenstrual period; another group which includes Blumenthal,[3] Detre,[4] Holler, Melicher, and Reiter,[5] Krutschenkoff,[6] Pölzl,[7] and Reinert,[8] have reported a compensatory increase in the number of red cells in the premenstrual phase followed by a decrease when the active flow begins. Four of the most extensive and representative studies, those of Leverton and Roberts,[9] Smith,[10] Reich and Green,[11] and Gumprich,[12] have shown no constant change which might be correlated with any part of the menstrual cycle.

We have, in the present study, determined erythrocyte counts and in addition the blood iron and blood copper values† for 10 normal women at various stages throughout the menstrual cycle. We feel justified in regarding the results of the blood iron and blood copper determinations as a more accurate index of the changing blood picture than erythrocyte counts and hemoglobin values determined by clinical hemoglobinometers.

METHODS

The subjects used in this work were young women between the ages of twenty and thirty-five. Most of them were laboratory technicians and nurses. All were in good health.

Samples for analysis were taken (1) on different days of the active flow, (2) at about five days after menstruation in the postmenstrual period, and (3) in the intermenstrual period midway between menstruations. The blood was drawn during a preabsorptive period at the same time each day to eliminate possible diurnal variations.

Copper and iron analyses were made on 5 c.c. samples of blood drawn from the cubital vein, and the iron results were checked by another iron method on a 0.5 c.c. sample. Details of the methods for copper[13] and for iron[14, 15] determinations have previously been described.

Special precautions were taken to obtain pure reagents, free from copper and iron. Acids, alkalies, and water were redistilled from glass and were tested frequently for contamination.

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†Blood iron and copper in all our work signifies iron and copper values as determined on whole blood unless otherwise stated.

Blood counts were made with standardized pipettes and counting chambers on blood drawn by cutaneous puncture of the lobe of the ear. Hemoglobin values were calculated from the blood iron content; iron constitutes 0.335 per cent of the molecular weight of hemoglobin.[16]

EXPERIMENTAL DATA

In Table I are recorded for each subject the part of the menstrual cycle during which the sample was taken, the red cell count, the iron content, the

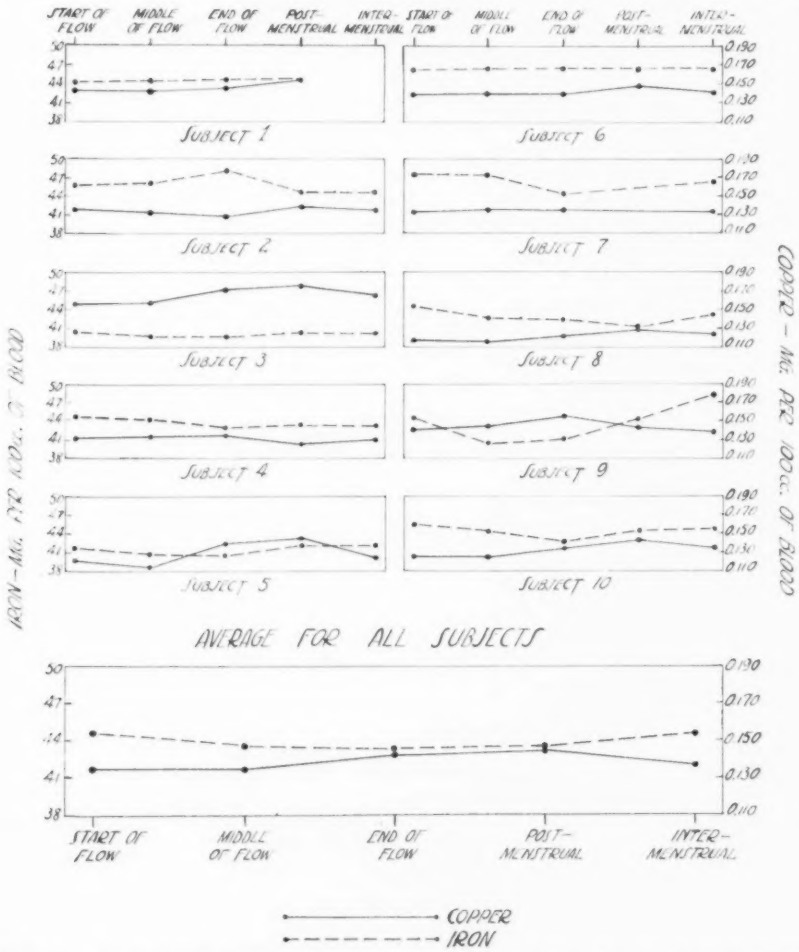

Chart 1.—Chart showing whole blood copper and iron values for ten normal women with relation to their menstrual cycles. Curves are charted for each individual and a composite curve representing averages of values for all subjects is included.

copper content, and hemoglobin in grams calculated from the blood iron. It is also stated in each case whether the flow was scanty, moderate, or excessive.

DISCUSSION

The results indicate that there is a slight decrease in the blood iron during the active menstrual discharge. The decrease is so slight, however, that it can scarcely be regarded as significant, the extremes in the average figures corresponding to little more than 1 mg. of iron per 100 c.c. of blood.

TABLE I

	RED CELLS PER C. MM.	HEMOGLOBIN GM. PER 100 C.C.	IRON MG. PER 100 C.C.	COPPER MG. PER 100 C.C.
Subject 1 Period—5 days in duration Quantity—normal				
First day of flow	4,540,000	13.21	44.25	0.143
Middle of flow	5,120,000	13.29	44.54	0.142
End of flow	4,450,000	13.46	45.11	0.149
Postmenstrual (5 days after flow)	4,480,000	13.43	45.00	0.153
Intermenstrual	--------	----	----	----
Subject 2 Period—5 days in duration Quantity—normal				
First day of flow	4,700,000	13.75	46.08	0.137
Middle of flow	5,130,000	13.91	46.61	0.132
End of flow	4,700,000	14.46	48.45	0.130
Postmenstrual (5 days after flow)	4,670,000	13.32	44.62	0.140
Intermenstrual	4,290,000	13.38	44.82	0.136
Subject 3 Period—5 days in duration Quantity—normal				
First day of flow	4,540,000	12.12	40.63	0.156
Middle of flow	4,350,000	11.89	39.85	0.159
End of flow	4,420,000	11.90	39.88	0.171
Postmenstrual (5 days after flow)	4,830,000	12.09	40.53	0.176
Intermenstrual	3,940,000	12.06	40.40	0.166
Subject 4 Period—4 days in duration Quantity—normal				
First day of flow	4,670,000	13.29	44.54	0.133
Middle of flow	4,420,000	13.14	44.02	0.134
End of flow	4,450,000	12.87	43.12	0.136
Postmenstrual (5 days after flow)	4,480,000	13.01	43.59	0.127
Intermenstrual	4,510,000	12.93	43.33	0.130
Subject 5 Period—6 days in duration Quantity—excesssive				
First day of flow	4,670,000	12.51	41.92	0.122
Middle of flow	4,580,000	12.22	40.96	0.114
End of flow	4,510,000	12.18	40.82	0.141
Postmenstrual (5 days after flow)	4,740,000	12.64	42.36	0.146
Intermenstrual	4,510,000	12.65	42.38	0.125
Subject 6 Period—3 days in duration Quantity—scanty				
First day of flow	4,700,000	13.79	46.22	0.141
Middle of flow	4,480,000	13.88	46.50	0.141
End of flow	4,380,000	13.86	46.45	0.141
Postmenstrual (5 days after flow)	4,480,000	13.83	46.35	0.149
Intermenstrual	4,610,000	13.78	46.18	0.143
Subject 7 Period—6 days in duration Quantity—normal				
First day of flow	4,740,000	14.26	47.78	0.133
Middle of flow	4,670,000	14.12	47.30	0.137
End of flow	4,740,000	13.18	44.16	0.137
Postmenstrual (5 days after flow)	--------	----	----	----
Intermenstrual	4,640,000	13.93	46.67	0.134

TABLE I—CONT'D

	RED CELLS PER C. MM.	HEMOGLOBIN GM. PER 100 C.C.	IRON MG. PER 100 C.C.	COPPER MG. PER 100 C.C.
	Subject 8			
	Period—4 days in duration			
	Quantity—normal			
First day of flow	4,800,000	13.31	44.58	0.118
Middle of flow	4,320,000	12.78	42.82	0.117
End of flow	4,770,000	12.71	42.59	0.124
Postmenstrual (5 days after flow)	4,350,000	12.33	41.33	0.129
Intermenstrual	4,380,000	12.92	43.31	0.124
	Subject 9			
	Period—3 days in duration			
	Quantity—excessive			
First day of flow	3,940,000	13.24	44.38	0.141
Middle of flow	3,680,000	12.15	40.71	0.144
End of flow	3,900,000	12.24	41.06	0.154
Postmenstrual (5 days after flow)	4,640,000	13.19	44.18	0.144
Intermenstrual	4,890,000	14.52	48.66	0.137
	Subject 10			
	Period—4 days in duration			
	Quantity—slightly excessive			
First day of flow	4,450,000	13.56	45.44	0.127
Middle of flow	4,030,000	13.21	44.27	0.125
End of flow	4,420,000	12.78	42.84	0.133
Postmenstrual (5 days after flow)	4,350,000	13.23	44.33	0.143
Intermenstrual	4,480,000	13.39	44.87	0.134
	Average for all subjects			
First day of flow	4,580,000	13.30	44.58	0.1351
Middle of flow	4,480,000	13.06	43.76	0.1345
End of flow	4,470,000	12.96	43.45	0.1415
Postmenstrual (5 days after flow)	4,560,000	13.01	43.59	0.1452
Intermenstrual	4,470,000	13.28	44.51	0.1365

The averages of the erythrocyte counts likewise show only small variations, the difference between the highest and lowest of the average counts being 110,000.

Looking at the subjects individually there is also a remarkably small variation in blood iron values. In the majority of the subjects the extremes of variation did not exceed 2 mg. and in every case except one, the variation was less than 4 mg. of iron and less than 1 gm. of hemoglobin per 100 c.c. of blood. Duckles and Elvehjem[17] have recently reported an average hemoglobin variation of 0.94 grams during the menstrual cycle in a series of seven women. It appears that the individuals showing the most appreciable drops in blood iron during the flow are those who have lost the largest amounts of blood during their menstrual period. The blood loss in menstruation may be considerable, as has recently been shown by Barer, Fowler, and Baldridge.[18] Their results, based on determinations made during menstruation in 50 normal women, indicate that the blood loss varies from 3.84 to 78.40 mg. of iron per menstrual period Assuming that the iron was derived entirely from the hemoglobin, which assumption is correct for all practical purposes, these figures represent 1.146 to 23.403 grams of hemoglobin lost per period, which is roughly equivalent to 9.39 to 207.28 c.c. of blood, with an average of 36.70 c.c. loss per menstrual period.

It seems correct to assume that in most individual cases and in averages of a representative number of cases, the iron, hemoglobin, and erythrocyte values show no decided waves coinciding with phases of the normal menstrual cycle. In a minority of cases where the periodic blood loss is far greater than the average, there may be an appreciable lowering of the blood iron and a mild anemia. The fact that Reich and Green[11] found no increase in the number of reticulocytes after the menstrual flow need not be considered contradictory to the finding of an occasional lowered blood iron, because the anemia in such instances is too mild and transitory to demand increased hematopoiesis to offset it.

The blood copper values show comparatively little change during the menstrual cycle. The averages disclose a very small increase in blood copper

TABLE II

CASE NO.	RELATION TO ONSET OF MENSTRUAL FLOW	COPPER, MG. PER 100 C.C.	IRON, MG. PER 100 C.C.	RED CELLS PER C. MM.	HEMOGLOBIN,* GM. PER 100 C.C.
1	20 hours before	0.134	44.87	4,480,000	13.39
	5 days after	0.143	44.33	4,352,000	13.23
2	14 hours before	0.150	44.35	4,760,000	13.24
	14 days after	0.147	45.28	4,830,000	13.51
3	24 hours before	0.127	43.54	4,768,000	13.00
	14 days after	0.133	42.44	4,672,000	12.66
4	2 hours before	0.120	43.77	4,480,000	13.06
	14 days after	0.130	45.67	4,224,000	13.63
5	20 hours before	0.129	46.92	4,576,000	14.01
	14 days after	0.121	47.10	4,832,000	14.06
6	16 hours before	0.149	44.27	4,290,000	13.21
	14 days after	0.137	45.09	4,608,000	13.45
7	8 hours before	0.125	41.88	4,480,000	12.50
	14 days after	0.148	43.41	4,360,000	12.95

*Hemoglobin calculated from the Butterfield factor[16] by the formula mg. of iron per 100 c.c. blood divided by 3.35 equals grams of hemoglobin per 100 c.c. blood.

at the end of the period of flow and in the postmenstrual phase, but the increase is too slight to justify the existence of hypercupremia. At its highest it is well within the physiologically normal average range previously established in our series on normal adults.[13] In Case 3 the copper values were somewhat increased. This subject, however, had the lowest blood iron in the group, the figure falling below 40 mg. per 100 c.c. of blood, and indicating a mild anemia. We have previously shown[13] that in anemia the blood iron goes down and the blood copper rises. Hence the increased copper in the above case is very likely due to the anemia and probably has no special relationship to menstruation.

Sarata[19] has reported in 4 cases a very marked increase in copper in blood from women taken within twenty-four hours preceding the onset of menstruation as compared with samples taken midway between menstrual periods. We have accordingly performed another series of analyses on a

small group comparing the copper and iron values of blood samples taken less than twenty-four hours preceding the onset of menstruation with samples taken in the intermenstrual period. The results are recorded in Table II.

On the basis of the 7 cases cited here no appreciable change can be noted in either the blood copper or iron in a comparison of values on the same subjects taken in the twenty-four-hour period preceding menstruation and in the intermenstrual period.

SUMMARY

Blood iron and copper determinations, red cell counts and hemoglobin determinations were made on a series of women in various periods of the menstrual cycle.

No significant changes could be noted which coincided with any definite phase of the menstrual cycle, although a slight decrease in iron and hemoglobin was noted in the period of menstrual flow followed by a slight rise in blood copper in the postmenstrual period. We have found no increase in blood copper within the twenty-four hours preceding the onset of menstruation.

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THE INFLUENCE OF THE QUANTITY OF SERUM BILIRUBIN ON THE TYPE OF VAN DEN BERGH REACTION*

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MUCH work has been done to explain the difference in the behavior of the serum bilirubin of hemolytic jaundice and that of obstructive jaundice with the van den Bergh reaction.

In general the attempt has been made to show that the two types of reactions could be explained by differences in the chemical or physical state of the bilirubin in the two types of jaundice. Van den Bergh[1] himself proposed these two possibilities. As to chemical differences, van den Bergh[2] and Andrewes[3] have shown that the serum bilirubin giving the direct reaction oxidizes more easily than the serum bilirubin giving the indirect reaction. Collinson and Fowweather[4] believe from their work that the bilirubin giving the "direct" reaction is probably an ammonium salt of bilirubin, while the "indirect" type of bilirubin is the free acid. Barron[5] was unable, however, to confirm their experiments.

Regarding differences in the physical states of the two types of bilirubin, van den Bergh[2] found that the "direct" bilirubin was more easily adsorbed by protein precipitated by alcohol than is the case with the "indirect" bilirubin. Grunenberg[6] has reported differences in the chloroform solubilities of the bilirubin occurring in the two types of jaundice. Hoover and Blankenhorn[7] and Leschke[8] have reported that the bilirubin of obstructive jaundice differed from that of hemolytic jaundice in being readily dialyzable through collodion membranes. Gregory and Andersch[9] have reported experiments which show that neither the serum bilirubin of obstructive jaundice nor the bilirubin which has passed through the liver cells (gall bladder bile) or through the kidney is filtrable or dialyzable through collodion membranes which will not permit the passage of protein.

Working with xanthochromic spinal fluid, Vaughan and Hubbard[10] concluded that the type of reaction depends upon the quantitative relationship between the pigment and protein. They found with material of low protein concentration that when the bilirubin exceeded 0.3 mg. per cent the van den Bergh reaction became direct. They believe, therefore, that it is unnecessary to consider that passage through the liver cells is the determining factor which changes the "indirect" to the "direct" type of bilirubin.

Some of our experiments have yielded results which we believe show that the quantity of the serum bilirubin plays no part in determining whether the bilirubin will give the direct or the indirect type of van den Bergh reaction.

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METHODS

Blood samples were obtained from the cords of 11 babies at birth. These bloods were analysed for serum bilirubin according to the Gibson and Goodrich modification[11] of the van den Bergh reaction. Most of the babies were observed for three days, and a urine specimen obtained three to five days after birth.

Gall bladder bile obtained at autopsy was filtered and diluted with pooled serum, to make a series of sera containing from 1.8 mg. per cent (the diluting serum, van den Bergh indirect) to 12.5 mg. per cent of bilirubin. These sera were analysed by the quantitative van den Bergh test as modified by Gibson and Goodrich.

TABLE I

BABY	CLINICAL JAUNDICE	VAN DEN BERGH MG. %	TYPE OF VAN DEN BERGH	COLOR OF URINE	GMELIN TEST ON URINE
O	+	26.0	Indirect	Straw-colored	Negative
G		9.6	Indirect	Straw-colored	Negative
E		12.2	Indirect	Water clear	Negative
W	+	13.0	Indirect	Straw-colored	Negative
Li	++	28.8	Indirect	Straw-colored	Negative
K	+	26.2	Indirect	Straw-colored	Negative
S	+	22.2	Indirect	Straw-colored	Negative
L		7.6	Indirect	Straw-colored	Negative
Mi		2.8	Indirect	Straw-colored	Negative
W		11.4	Indirect	Straw-colored	Negative
M		18.5	Indirect	Straw-colored	Negative

TABLE II

SERUM DILUTION	VAN DEN BERGH MG. %	TYPE OF VAN DEN BERGH
Pooled serum	1.8	Indirect
1	3.0	Direct
2	4.1	Direct
3	5.2	Direct
4	6.2	Direct
5	7.2	Direct
6	8.0	Direct
7	9.4	Direct
8	10.2	Direct
9	11.2	Direct
10	12.5	Direct

Sera were prepared as follows: a few drops of filtered bile were added to the pooled serum. This resulted in a serum which gave a "direct" van den Bergh of 12.5 mg. per cent. Sera 1 to 10 were obtained by diluting this with the pooled serum with van den Bergh of 1.8, "indirect."

RESULTS

Our results are listed in the accompanying tables. Reference to Table I shows that bilirubin of the serum of cord blood gives unusually high values for indirect van den Bergh reactions. With the exception of babies G, W, and K, whose cord blood serum gave a very faint trace of color with the "direct" procedure, all of the values obtained were of the "indirect" type.

In Table II is shown the results of subjecting serum dilutions of filtered bile to the van den Bergh test. All of these diluted and "artificially" jaundiced sera gave direct van den Berghs, with values ranging from 3.0 to 12.5 mg. per cent.

A van den Bergh value as high as 28.8 mg. per cent "indirect" in the cord blood and as low as 3.0 mg. per cent "direct" in artificially jaundiced sera, allows one to conclude that the results of Vaughan and Hubbard on spinal fluid are not applicable to blood.

We did not estimate the serum proteins on the cord blood. Peters and Van Slyke,[12] quoting Bakwin and Rivkin[13] and Mello-Leitao,[14] state that the serum of infants "contains considerably less protein than that of adults." Although neither of the original papers dealt with cord blood of newborns, blood from babies as young as twelve days was analysed for protein.

Assuming that these low plasma proteins occur in cord blood of newborns, it invalidates further the conclusions of Vaughan and Hubbard so far as blood bilirubin is concerned. In as much as it is their conclusion that the greater the amount of bilirubin in proportion to the protein in solution, the more likely a direct van den Bergh reaction will result.

The question naturally arises as to why the van den Bergh reaction of 1.8 mg. per cent "indirect" of the pooled sera became "direct" after the addition of the gall bladder bile. The nature of the substance in bile responsible for this is being further investigated.

CONCLUSIONS

The quantity of bilirubin in serum is not the factor which determines whether it will give the "direct" or "indirect" van den Bergh reaction. It appears unlikely that the quantitative interrelationship between serum bilirubin and protein is the determining factor.

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ELECTROCARDIOGRAPHIC STUDIES DURING ANESTHESIA WITH INTRAVENOUS BARBITURATES*

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TOXIC effects from anesthetic drugs are dramatically displayed when the respiratory or circulatory functions are seriously disturbed. A number of procedures for promptly restoring these vital activities to normal have been advanced. The anesthetist is not without adequate methods to contend with respiratory arrest but is afforded little security with his efforts when confronted with severe cardiac disturbances. The inability to effect a favorable result for not a few of these patients has focused attention upon the causes of such complications. Consequently, the effects of various anesthetic agents on the heart have been the subject of many recent investigations.

Kurtz and his associates[1] reported an extensive clinical study of the electrocardiographic changes during anesthesia with most of the anesthetic agents in common use. Arrhythmias and other cardiac irregularities were not uncommon. Similar studies have been completed in other clinics,[2, 3] but the changes during anesthesia from the intravenous use of soluble barbituric acid derivatives have not been included.

Gruber[4] first presented the results from electrocardiographic studies with barbituric acid derivatives. In discussion at the annual Congress of Anesthetists, October, 1936, he reported preliminary observations which indicated that the thio derivatives of barbituric acid employed as anesthetic agents consistently produced cardiac irregularities in laboratory animals. These disturbances were accentuated if the thio-barbiturates were combined with morphine. Barbiturates which did not contain a sulfur radical produced no such cardiac disturbances. Since then Gruber has studied this phenomenon more extensively and has found a similar reaction in practically every instance for different species of laboratory animals. Kohn, working in the experimental division of the Abbott Laboratories, found no constant electrocardiographic changes in animals anesthetized with sodium thio-pentobarbital.[5] Reme and others recently reported electrocardiographic studies during pernocton and evipan anesthesia in animal experiments.[6] They found alterations in the electrocardiogram during anesthesia with these agents, but attributed the changes to anoxemia rather than to the anesthetic agents. Hafkesbring and MacCalmont[7] studied electrocardiographic changes during anesthesia with nembutal, amytal, and barbital in the dog and cat. They observed no outstanding irregularities in cardiac rhythm or conduction.

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The increasing popularity of intravenous anesthesia with the barbituric acid derivatives, especially the thio derivatives, and the electrocardiographic changes observed in laboratory animals warranted a clinical study by means of the electrocardiogram while these drugs were employed.

The drugs used in this study were sodium thio-pentobarbital (pentothal or sodium thionembutal), sodium isoamyl ethyl thio-barbiturate (sodium thio-amytal),* and sodium N-methylcyclohexenyl-methyl barbituric acid (evipal soluble). The latter two were employed in 10 per cent aqueous solution. A 5 per cent aqueous solution of pentothal was used. There was a total of 17 anesthesias for 12 patients. Three of the patients were anesthetized at different times with different agents.

Patients were chosen at random from a general surgical ward and had only the routine physical examination and laboratory study. The surgical procedures were of a minor nature but required obtundation of pain (e.g., removal of a painful dressing or manipulation of arthritic extremities). Guedel's signs of anesthesia were followed for the classification of degrees of narcosis.

In eight cases preanesthetic medication with morphine sulfate $\frac{1}{8}$ to $\frac{1}{4}$ gr. (0.0075 to 0.015 gm.) or atropine $\frac{1}{150}$ to $\frac{1}{100}$ gr. (0.0004 to 0.0006 gm.) or a combination of these two drugs was given subcutaneously one hour before anesthesia. Ephedrine sulfate and adrenalin hydrochloride were given intravenously to effect (rise in systolic blood pressure and an elevation in pulse rate) at the height of the anesthesia in an attempt to sensitize the myocardium to any possible minimal effect of the anesthetic agent on the heart. Seven cases received no drugs other than those used to produce narcosis.

Studies were completed by securing a preliminary or normal electrocardiographic tracing, using the three leads. The barbiturate was then given intravenously until surgical anesthesia was obtained while Lead II was continually being recorded. Tracings of Lead II were completed for a short time after the height of anesthesia was reached and in some cases during the surgical procedure. All three leads of the electrocardiogram were taken during recovery until the patient had reached the status of rational response to questioning.

The cases in which pentothal was employed demonstrated by electrocardiographic tracings only a slight increased heart rate. There were no QRS or T-wave abnormalities.

CASE REPORTS

G. P., (Fig. 1), a fifty-two-year-old male. History and physical examination were essentially negative, except for local surgical condition (hemorrhoids). On admission, patient's blood pressure was 110/60, pulse rate 80, W.B.C. 9,500, R.B.C. 4,500,000, hemoglobin 85 per cent. Pentothal was given on two successive days to facilitate changing a painful posthemorrhoidectomy dressing. For the first anesthetic no preliminary medication was employed, but for the second, morphine sulfate ¼ gr. (0.015 gm.) was administered an hour before an-

*Parke, Davis & Co., Detroit, Mich.

esthesia. An electrocardiogram, prior to anesthesia, revealed a right bundle-branch block with a deep S-wave in Lead I and a QRS of 0.12 second. No appreciable change occurred in the bundle-branch block or the P-R interval during anesthesia on either occasion. The only recorded difference was a slight and temporary increase in pulse rate.

A. C. (Fig. 2), a white male, fifty years of age, studied one month after operation for relief of acute intestinal obstruction. There was a well functioning ileostomy. Patient's history

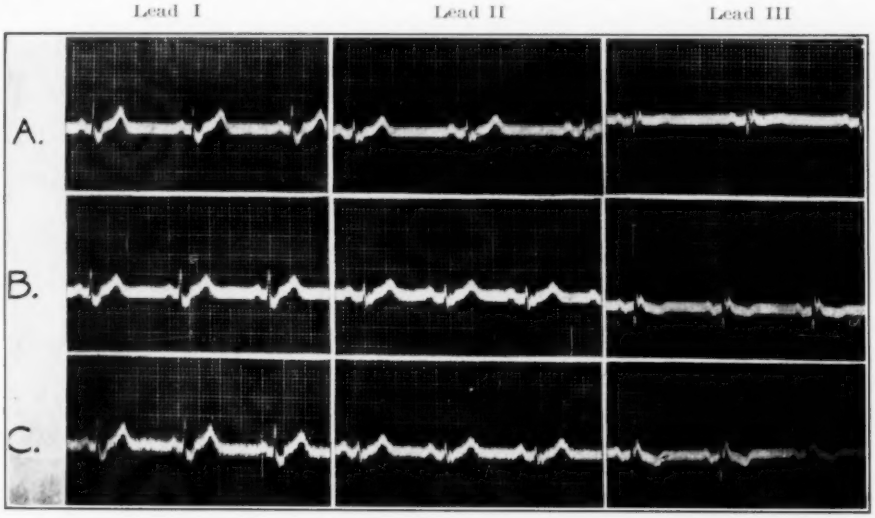

Fig. 1.

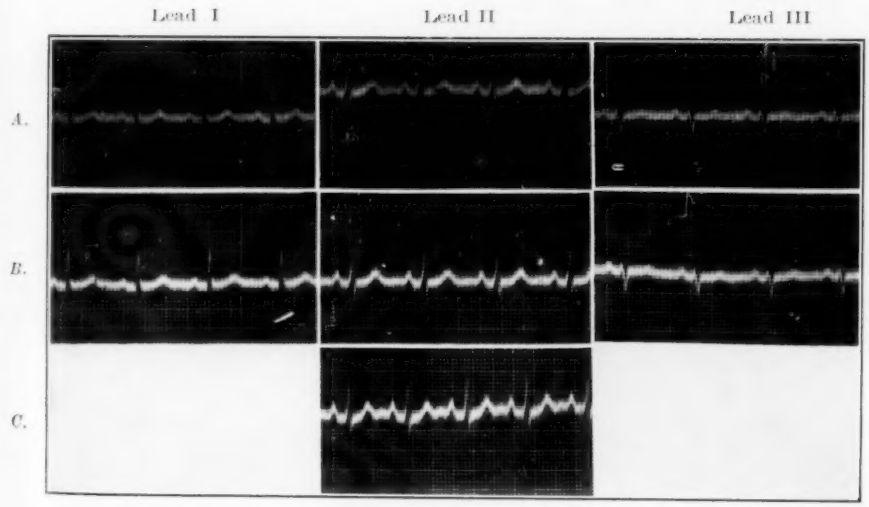

Fig. 2.

and physical condition essentially negative, except for the local surgical condition and a moderate secondary anemia. Blood pressure 140/70, pulse rate 88, temperature (rectal) 99.4° F., R.B.C. 3,500,000, W.B.C. 14,600, hemoglobin 80 per cent. One and one-half hours prior to anesthesia, morphine sulfate 1/6 gr. (0.01 gm.) was given subcutaneously. The blood pressure was 110/60 and the pulse rate 70 immediately before anesthesia. Two hundred and fifty milligrams of pentothal were sufficient to induce surgical anesthesia. At the height of narcosis one minim of epinephrine hydrochloride (1/1000) in 1 c.c. of sterile saline was given intravenously. Within forty-five seconds the blood pressure increased from 110/70 to 180/90

and the pulse rate from 70 to 124. Five minutes later the blood pressure was 90/50 and the pulse rate 78. During the entire period of anesthesia, there were no changes in the electrocardiographic tracings other than an increase in the heart rate.

J. M. (Fig. 3), a fifty-year-old white male. History and physical examination essentially negative, except for a painful hypertrophic arthritis of the right wrist. Temperature was 99° F., pulse rate 80, blood pressure 150/90, R.B.C. 3,900,000, W.B.C. 10,500, hemoglobin 90 per cent. No premedication was given. At onset of anesthesia the blood pressure was 160/100, pulse rate 90. Six hundred milligrams pentothal were used to produce the desired anesthesia. This was followed immediately by the intravenous administration of one and one-half minim adrenalin (1/1000) in 1.5 c.c. of normal saline. One minute later, the blood pressure reached a maximum height of 195/105. The pulse rate was then 120. In eight minutes the blood pressure was 145/95 and the pulse rate 96. During this interval, the arthritic wrist was manipulated. Throughout the entire procedure the electrocardiogram demonstrated no change except an increased heart rate.

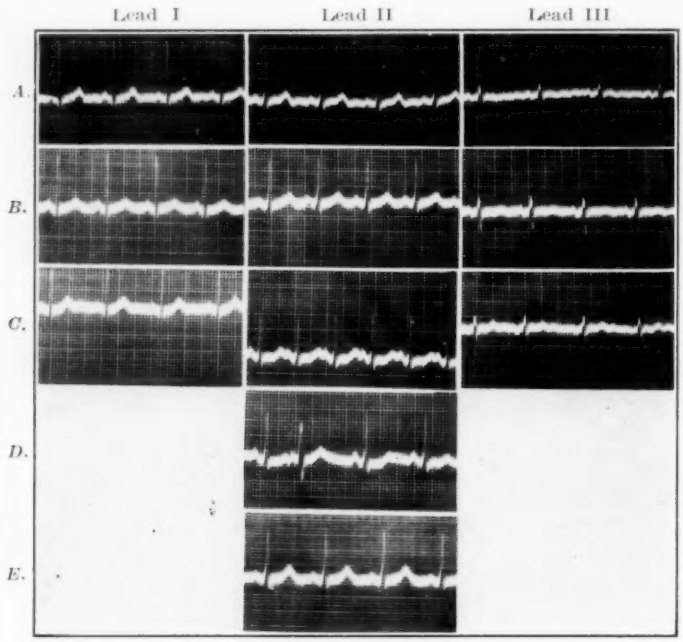

Fig. 3.

Eight electrocardiographic studies were completed during the intravenous administration of thio-ethamyl. The electrocardiograms revealed no changes in the QRS complex, P-R interval, or T-wave. There was noted only an increase in heart rate.

R. M. (Fig. 4), fifty-two-year-old white male. History and physical examination entirely negative, except for local surgical condition, cellulitis of the right foot, which had been incised and drained two days previously. Temperature 101° F., R.B.C. 4,200,000, W.B.C. 20,100. Blood pressure 160/100, and pulse rate 80 before anesthesia. No premedication was employed. Electrocardiograms before anesthesia revealed a regular sinus rhythm with ventricular premature contractions and coupling. The QRS was slurred in Leads II and III. Five hundred milligrams of thio-ethamyl were given intravenously to produce surgical anesthesia. Lead II of the tracing, taken during induction, showed a rate increased to 100, but the ventricular premature contraction and coupling remained unchanged for four minutes

during the anesthesia and then suddenly disappeared and a normal rhythm was observed during the remainder of the anesthesia and throughout the recovery period. There were no changes in the QRS or P-R interval.

M. M. (Fig. 5), colored female, aged thirty-four years. History and physical examination were essentially negative, except for the local surgical condition. A human bite on the

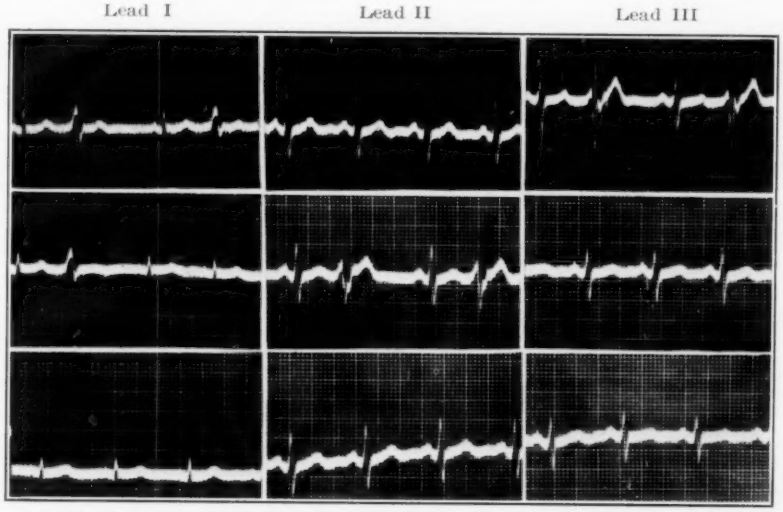

Fig. 4.

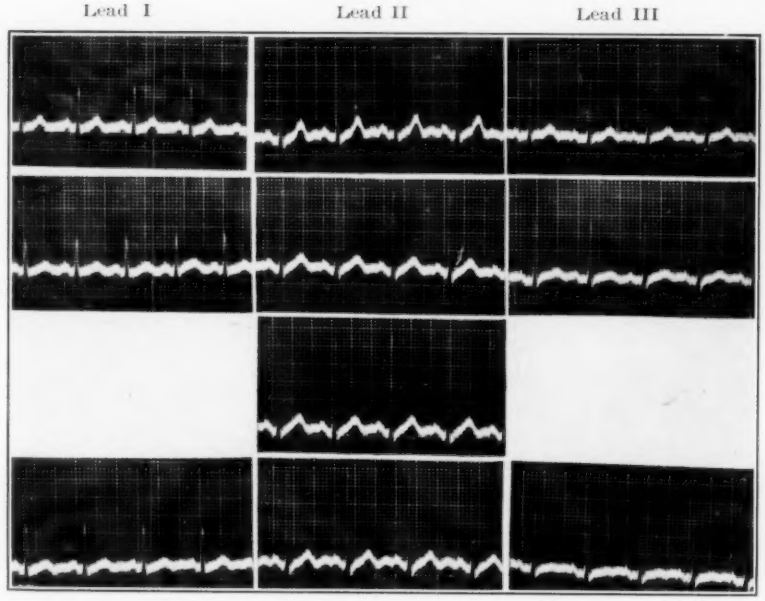

Fig. 5.

left thumb had been incised and drained one day before. Blood pressure was 125/82, pulse rate 84, temperature 98.4° F. No preliminary medication was given. Twelve hundred and fifty milligrams of thio-ethamyl were required to produce surgical anesthesia. Blood pressure was then recorded 112/70, pulse rate 120. Twelve and one-half milligrams of ephedrine sulfate in 5 c.c. of sterile water were injected intravenously. Within forty seconds there was an increase in blood pressure to 155/85. Three and one-half minutes later the blood pressure

was at the original level. During this interval drains in the infected thumb were replaced. The electrocardiogram revealed no abnormalities at any time during the procedure except an early, transient tachycardia.

E. M. (Fig. 5), white male, aged fifty-two years. History of chronic alcoholism. Admission for cellulitis of the right forearm. Temperature 98.8° F., pulse rate 90, blood pressure 115/80. R.B.C. 3,730,000, W.B.C. 12,900, hemoglobin 65 per cent. Patient received subcutaneous morphine sulfate 1/6 gr. (0.01 gm.) and atropine 1/150 gr. (0.0004 gm.) one hour before anesthesia. Seven hundred milligrams of thio-ethamyl produced surgical anesthesia. Blood pressure was 110/75 and pulse rate 90 at this time. Twenty-five milligrams of ephedrine sulfate diluted with 10 c.c. of sterile water were given intravenously within two minutes. Blood pressure was then 140/85 and the pulse rate 100. Lead II of electrocardiogram taken during and after the procedure did not deviate from the normal.

In the same manner three patients were given evipal soluble intravenously to produce surgical narcosis. The first received no premedication, the second received morphine 1/6 gr. (0.01 gm.) as a preliminary sedative, the third morphine sulfate 1/6 gr. (0.01 gm.) and atropine 1/150 gr. (0.0004 gm.). Electrocardiographic tracings in these three cases were normal.

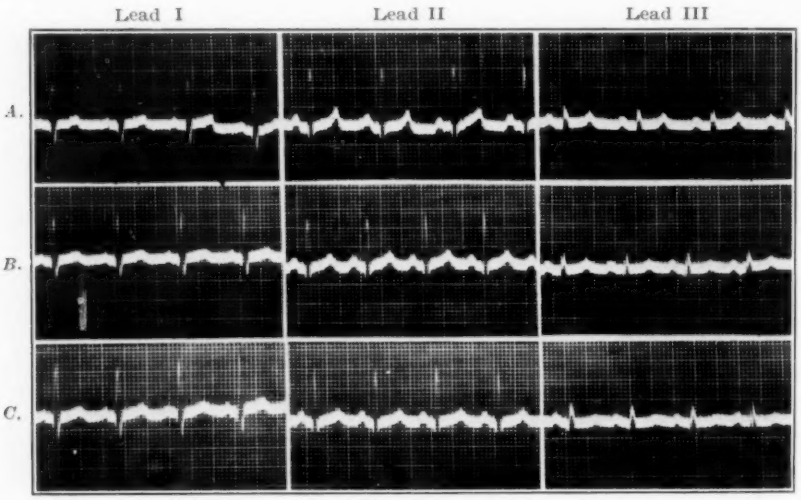

Fig. 6.

In every instance where atropine was employed as a preanesthetic drug in conjunction with the barbiturates, the return of the ventricular rate to normal was delayed.

SUMMARY

In seventeen clinical trials the electrocardiogram revealed no deviation from the normal in the QRS, P-R interval, or T-wave when either sodium thiopentobarbital (pentothal), sodium isoamyl ethyl thio-barbiturate (sodium thioethamyl), or sodium N-methylcyclohexenyl-methyl barbituric acid (evipal soluble) was employed intravenously to produce surgical narcosis.

Preliminary medication with morphine sulfate, with atropine or with the two combined, affected the heart by prolonging an increased rate. Patients receiving atropine showed this effect constantly.

Epinephrine hydrochloride or ephedrine sulfate given intravenously in small amounts at the height of thio-barbiturate anesthesia did not precipitate cardiac irregularities.

Cardiac irregularities already present were not altered by the drugs used in this investigation.

This investigation, supported by a grant from Parke, Davis & Co., Detroit, Mich., was completed under the direction of Dr. Arthur C. DeGraff, Department of Therapeutics, and Dr. E. A. Rovenstine, Department of Anesthesia.

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THE INCIDENCE OF TUBERCULOSIS IN A NEW YORK CITY DIABETIC CLINIC*

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IN RECENT years there have been an increasing number of reports on the association of pulmonary tuberculosis and diabetes. The paper by Root[1] in 1934 reviews the literature so thoroughly that it seems unnecessary to repeat what he has already said. Since then a number of other articles have appeared on the subject.[2-6] King[7] has pointed out that, although tuberculosis complicating diabetes has decreased during the past fifty years, it is still approximately five times more frequent in diabetics than in the population as a whole. Most of the reports have come from tuberculosis sanatoriums. We have studied the occurrence of tuberculosis in the diabetic patients attending the Diabetic Clinic of the Third Medical Division (New York University), Bellevue Hospital, and the Diabetic Clinic of New York University College of Medicine. The total number of diabetics attending these clinics is, at present, 748. The observations were made over a period of seven years.

Of the 748 patients, 33 had active pulmonary tuberculosis, an incidence of 4.3 per cent. Table I gives the sex and age of these patients, the time of onset of the diabetes and the tuberculosis, and the diets and amounts of insulin needed. Thirteen of the patients were females, twenty-one were males. The age at which the onset of tuberculosis occurred varied from nineteen to seventy-nine years; seventeen were under fifty years of age. Excluding the

*From the Diabetic Clinics of the Third Medical Division (New York University), Bellevue Hospital, and the New York University College of Medicine Clinic.
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one patient who was seventy-nine, which seems to us exceptional, the average age was 47.8 years. This is a somewhat higher age incidence for the onset of tuberculosis than occurs in the population as a whole. In 29 cases the onset of the diabetes preceded, as far as we could ascertain, any signs or symptoms of tuberculosis. In 3 cases the diagnosis was made simultaneously. The diets used in these patients were high in carbohydrate, moderate to high in fat,

TABLE I

CLINIC DIABETIC PATIENTS WITH TUBERCULOSIS

CASE NO.	SEX	AGE	DIAGNOSIS OF DIABETES	DIAGNOSIS OF TBC.	DIET C P F	DAILY INSULIN UNITS	CLASSIFICATION OF TBC.	COURSE
1	F	31	1929	1928	150-70-140	20	III-C	Died, 1930
2	F	43	1921	1929	140-60- 90	35	III-C	Died
3	F	19	1928	1929	125-60-130	15	II-C	Sanatorium
4	M	39	1927	1930	160-75-120	30	III-C	Died
5	F	33	1929	1930	150-65-115	27	III-B	Died
6	F	58	1917	1930	200-90-105	10	III-B	Oleothorax—doing well
7	M	62	1928	1930	180-70- 85	15	III-C	Died—perforated gastric ulcer
8	M	62	1926	1930	150-64-100	10	II-B	No follow-up
9	F	48	1924	1930	130-64-100	15	III-C	Died
10	M	48	1927	1930	150-65-120	10	III-B	Died
11	F	23	1929	1931	280-77-195	95	III-C	Died
12	M	62	1930	1931	150-60-100	40	III-C	Died
13	M	56	1926	1932	215-70-100	48	I-A	No follow-up
14	M	19	1929	1933	200-65- 85	120	II-B	No follow-up
15	M	40	1932	1933	150-70- 85	38	III-C	Died
16	M	51	1930	1933	350-80-120	95	III-B	Prognosis poor
17	M	40	1930	1934	250-90-110	75	III-B	Prognosis poor
18	F	48	1933	1934	180-70- 85	20	II-B	Sanatorium
19	M	65	1933	1934	200-75- 65	10	III-B	Prognosis poor
20	M	56	1931	1934	300-90-100	18	III-B	No follow-up
21	F	48	1917	1930	150-65- 85	25	II-A	Arrested
22	F	52	1928	1934	200-65- 85	35	III-B	Prognosis poor
23	F	61	1931	1934	150-65- 85	35	III-B	Died
24	M	46	1935	1935	250-65- 85	55	III-B	Prognosis good
25	F	26	1929	1935	300-65-130	70	III-C	Died
26	M	51	1930	1923	200-65-120	40	II-A	Arrested
27	M	69	1933	1936	150-65- 85	12	I-A	No follow-up
28	M	46	1934	1936	325-80- 85	105	III-B	Sanatorium
29	M	79	1934	1936	200-70- 85	20	III-C	Died
30	M	64	1927	1936	180-70- 85	60	II-A	Arrested
31	M	58	1936	1936	180-65- 85	50	I-A	Arrested
32	F	46	1934	1935	250-65- 85	100	III-A	Arrested
33	M	32	1931	1931	250-70-100	65	III-B	Prognosis poor

and with a protein intake of 65 to 85 grams. All but one patient required insulin, and twenty-two of them required more than thirty units daily.

On admission the tuberculosis classification, according to the National Tuberculosis Association criteria, was as follows:

Stage I	3 cases	9.0 per cent
Stage II	7 cases	21.0 per cent
Stage III	23 cases	68.9 per cent

Treatment of the Tuberculosis.—Ten of the patients received pneumothorax. Two had, in addition, pneumolyses which were not successful. In one case there was hemoptysis which could not be controlled by pneumothorax or

phrenicotomy, and the patient finally died of a bronchiogenic spread. Five of the patients, during their hospital period, left against advice, and we have been unable to obtain any further information about them.

Course.—Of the 33 patients observed, 13 have died since they developed tuberculosis. Ten no longer attend the clinic, but as far as we know are still alive. Of the remaining 10, 5 are now attending the diabetic clinic, having been treated successfully for their tuberculosis. The remaining 5 are at present in sanatoriums. Three of the five patients attending the clinic are considered inactive.

Treatment of the Diabetes.—The combined incidence of these two diseases is interesting because of the effects of diabetes on the general nutrition of the individual and because of the importance of nutrition in tuberculosis. Diabetes, disturbing as it does not only the carbohydrate, protein, and fat metabolism of the individual, but also the metabolism of calcium, phosphorus, and certain of the vitamins, would naturally influence the course of a chronic infectious disease, such as tuberculosis. In considering the diabetic with tuberculosis, particular attention should be paid to the general state of nutrition of the individual and effort made not only to control the diabetes, but to provide the patient with a diet adequate in every respect. It is our policy in treating the diabetic patient with tuberculosis to give a diet which is ample in calories (2,500 to 3,000). The protein content averages from 65 to 85 grams, the carbohydrate from 150 to 300 grams, and the rest of the diet is made up with fat. In order to assure an adequate amount of vitamin A, part of this fat is given in the form of cod liver oil. In view of the high blood carotene that has been reported in diabetics,[8, 9] particularly when fed carotene, it seems wiser to give vitamin A as such rather than as its precursor.

As the tuberculous diabetic is apt to be a severe diabetic, we have found it advisable to feed the individual four times in twenty-four hours, rather than the usual three meals, as the latter leaves the patient with no outside source of energy for a period of fourteen hours, during which time he would be forced to burn his body stores. Insulin is given with each feeding in amounts sufficient to keep the patient approximately sugar free, and always acetone free. We advise the patients to eat at 8 A.M., 1 P.M., 6 P.M., and 11 P.M.

It is equally important that the intake of fluid and salt should be adequate. Diabetics become dehydrated very readily, particularly in the presence of an infection. We, therefore, advise the patients to drink ample amounts of water and to take a slight excess of salt. Normally an individual takes in approximately 4 grams of sodium chloride as such daily. In the severe tuberculous diabetic this should be increased to 6 grams a day or more if necessary. An adequate intake of calcium is assured by having the patient take at least three glasses of milk daily.

EXAMPLES OF CLINIC CASES

X-rays are shown on 3 of the clinic cases followed. The first of these, Case 14, a nineteen year-old boy, four years after developing diabetes was found to have minimal tuberculous infiltration when admitted to the hospital. Absolute rest was advised, but as the

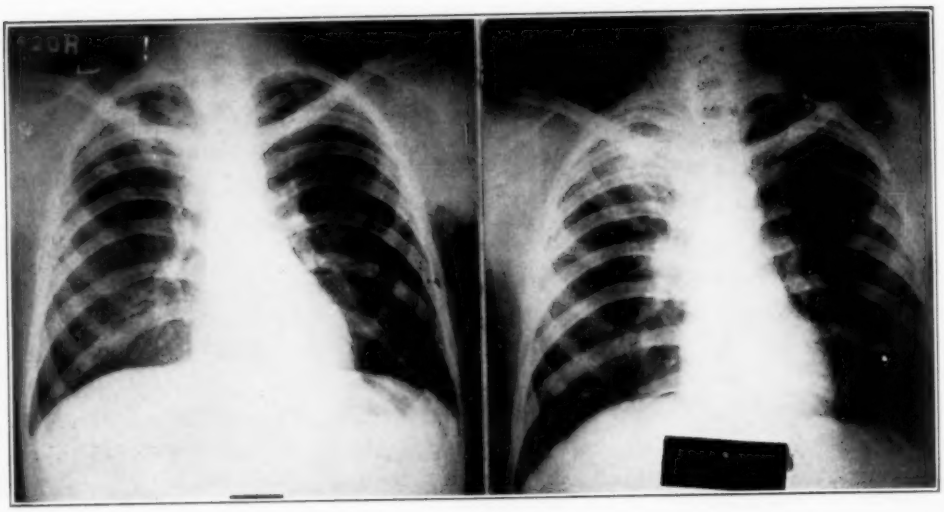

Fig. 1.—Case 14.

X-ray taken Jan. 9, 1933.
Early x-ray showing minimal degree of tuberculous infiltration at right apex.

X-ray taken Jan. 29, 1936.
Extension of tuberculous process to the third right anterior rib area.

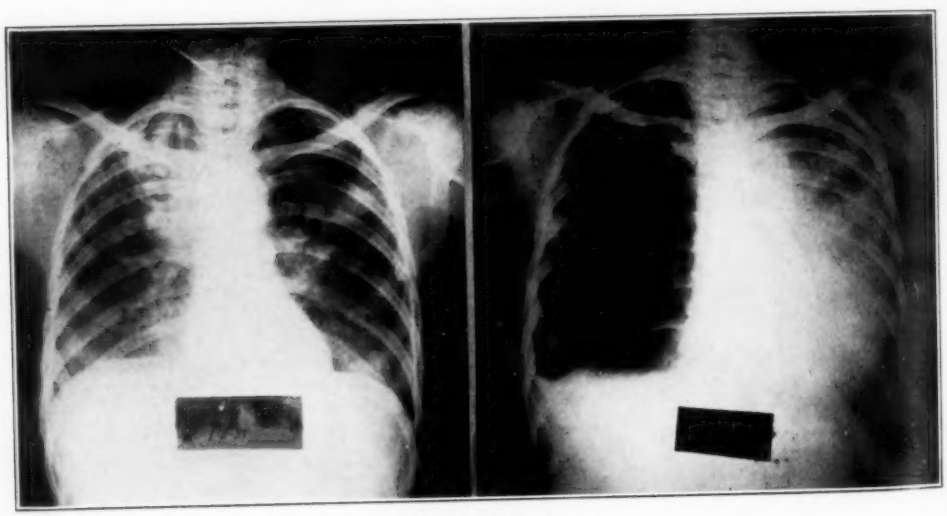

Fig. 2.—Case 8.

X-ray taken Sept. 11, 1935.
Widespread cavitation in upper third of right lung field with bronchiogenic spread to the base. Bronchiogenic spread in left midlung field with a small cavity.

X-ray taken Feb. 11, 1936.
Right hydropneumothorax with marked displacement of heart and mediastinum to the left. (Spontaneous bronchopleural fistula present on this side.) Widespread tuberculous bronchiogenic spread throughout left side.

patient was unruly he left the hospital against advice. A later x-ray shows a spread of the lesion, although the sputum was still negative. He was finally persuaded to go away to a sanatorium.

CASE 8.—A female, aged twenty-six years, with a history of diabetes of six years' duration. She had never cooperated in the treatment of her diabetes and had been admitted frequently in diabetic ketosis. After an absence from the clinic of four months, she was admitted to the hospital in profound ketosis and at this time was found to have advanced tuberculosis. Treatment consisted of pneumothorax on the right, although the lesion was bilateral. Pneumolysis was done but was unsuccessful, and the patient finally died.

CASE 24.—A forty-six-year-old male developed diabetes and tuberculosis apparently at the same time. When he was first seen, there was a large cavity in the left midlung field. Pneumothorax was given and the patient's course was satisfactory, the sputum becoming negative. He now attends the diabetic clinic and is receiving pneumothorax.

Hospital Group (Table II).—During the period of time that these patients were observed in the clinics, patients with diabetes and tuberculosis, who had

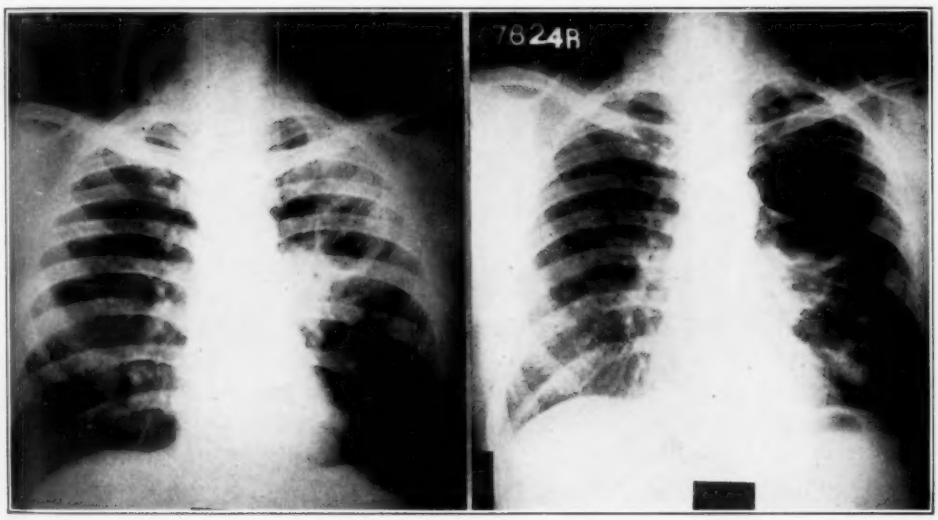

Fig. 3.—Case 24.

X-ray taken Dec. 30, 1935. Huge cavity in upper third of lung field with bronchiogenic dissemination from apex to fourth anterior rib.

X-ray taken March 18, 1937. Adequate pneumothorax collapse on the left.

never been previously treated in the clinic, were admitted to the Third Medical Division (New York University), Bellevue Hospital. These patients were either unaware of their diabetes, or the diagnosis having been made, had not bothered with any treatment. The total number of such patients from 1929 to 1936 was 21. The total number of diabetic patients admitted to the Third Medical Division during this same time was 986, giving a 2.5 per cent incidence of tuberculosis in the group of diabetic patients. All were in Stage III, far-advanced tuberculosis, when admitted. The average age was 48.3 years. All but 3 were over thirty years of age. In every case there was knowledge of the diabetes before the tuberculosis. Six of the patients became aware of the existence of both diseases when they entered the hospital. In 11 patients, although diabetic symptoms were present before admission, treatment for

both diseases began only after hospitalization. Of the 21 patients, 14 have died, and 2 are still in sanatoriums; one, although quite sick, attends the clinic. There are no follow-up records on the other four.

TABLE II

NONCLINIC DIABETIC PATIENTS WITH TUBERCULOSIS ADMITTED TO THE HOSPITAL

NAME	SEX	AGE	DIABETES DIAGNOSED	TBC. DIAGNOSED	DIET C P F	DAILY INSULIN UNITS	TBC. CLASSIFICATION	COURSE
A. K.	M	50	1925	1929	100-60- 90	0	III-C	Died
E. M.	F	40	1929	1929	160-70-140	60	II-C	Died
S. B.	M	27	1929	1930	196-90-160	42	III-C	Probably dead
P. G.	M	53	1925	1930	175-70-120	10	III-C	Died
J. M.	M	52	1920	1930	130-64-100	0	III-B	No follow-up
S. P.	F	60	1930	1932	Unable to eat		III-C	Died
J. F.	M	43	1929	1930	150-64-100	70	III-A	Prognosis poor
R. C.	M	48	1930	1932	200-65- 85	38	III-B	Died
A. H.	F	29	1928	1932	200-60- 85	13	III-C	Died
A. H.	F	48	1931	1931	150-64-100	27	III-C	Probably dead
E. M.	M	32	1932	1932	260-70-130	58	III-B	No follow-up
E. O.	F	65	1931	1932	Ketosis regime		III-C	Died
J. A.	M	43	1931	1933	230-70-155	48	III-C	Died
C. W.	M	68	1928	1933	Unable to eat		III-C	Died
M. S.	F	54	1926	1933	200-65- 85	25	III-C	Died
S. T.	M	33	1933	1933	250-65- 85	0	III-B	Sanatorium
J. F.	M	61	1929	1934	Unable to eat		III-C	Died
P. P.	M	38	1935	1935	180-70- 85	30	III-B	Prognosis bad
L. S.	M	46	1931	1935	200-75- 85	15	III-C	Died
J. S.	M	69	1933	1935	250-75- 85	0	III-C	Died
J. R.	M	58	1936	1936	250-65- 85	60	III-C	Died

Treatment and Course of the Tuberculosis in This Group.—Pneumothorax was tried on 6 patients, all unsuccessful because of adhesions and of the progress of the disease. Two of them left against advice and were not adequately followed. The remainder were given bed rest only. Since every case was in the far-advanced stage when they first entered, it is only natural to expect that the majority by this time have died.

DISCUSSION

Although the prognosis in diabetes and tuberculosis is still far from encouraging, the outlook need not be hopeless. This is borne out by the difference in the severity of the tuberculosis in the group of patients who were never properly treated for their diabetes and were admitted to the hospital for both diabetes and tuberculosis, as contrasted with the diabetic patient treated in the clinic who received adequate treatment early for both diseases. Several facts account for the better prognosis in the clinic group. Attending as they do a chronic disease clinic at regular intervals and being under medical supervision, the diagnosis is usually made early, which makes it possible to give the patient better care for both diseases. Additional evidence of this fact is that several of the patients in the clinic group who died were patients who did not attend the diabetic clinic regularly and who had been admitted to the hospital several times in diabetic ketosis, due to lack of adherence to diet and insulin. Other observers of these combined diseases have pointed out the fact that acidosis influences the prognosis unfavorably, as it increases the loss of

calcium from the body, causes dehydration, blood concentration and loss of base. Furthermore, the uncontrolled diabetic is in a poor state of nutrition, which would further reduce his resistance to infection.

It is in the clinic group that there is the greatest hope for the prevention of advanced tuberculosis. If the diabetic patient is manageable, it should be possible to have routine x-rays of the chest every six months. For the past three years, we have tried to x-ray routinely every diabetic patient attending the clinic, and up to date have been successful in x-raying 80 per cent of the cases. If this is done, the tuberculous involvement may be discovered in its minimal stages and adequate treatment may arrest the disease.

The same general principles that are followed in the treatment of the nondiabetic tuberculous patients are advocated for the diabetic patient. It is, however, essential in the diabetic that the diabetes be well controlled at all times. With late cases in which the diagnosis of tuberculosis is made when the disease is advanced, the prognosis, as our experience indicates, is hopeless even though all the modern methods of collapse therapy, including pneumothorax and pneumolysis, are utilized.

SUMMARY AND CONCLUSIONS

In two active diabetic clinics with a total registration of 748 patients, pulmonary tuberculosis was present in 4.5 per cent of the cases. Of the patients with pulmonary tuberculosis, 13 died during the period of observation, which covered a period of seven years.

The treatment of diabetes complicating tuberculosis is outlined, and the treatment of the tuberculosis in these cases is given.

Attention is again drawn to the importance of early diagnosis in these cases and of careful control of the diabetes.

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DETECTION OF CIRRHOSIS AND OTHER DISEASES OF THE LIVER BY LABORATORY TESTS*

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LABORATORY tests for the diagnosis and study of liver diseases are numerous but skepticism prevails concerning their reliability. The hesitancy with which such tests are utilized seems unjustified if one may accept the conclusions of numerous investigations. Although it is true that many procedures advocated have been found subsequently to have little merit, the value of others has been established. Among the newer laboratory aids that have been proposed for the study or detection of liver disease, the Takata-Ara test, the fractionation of serum proteins, and the determination of esterified and free cholesterol have not been studied as extensively or correlated as completely with other tests as is desirable for evaluation. The present investigation is an appraisal of the accuracy of these procedures as used in a large general hospital, compared with estimation of icterus index and bromsulfalein retention under similar conditions. The records of over 250 patients in whom bedside examination demonstrated or suggested liver involvement were studied. In 228 patients it was possible with reasonable certainty to establish or exclude the presence of liver disease, and the data have been analyzed with the object of correlating the results of laboratory tests with clinical or pathologic findings.

The series included decompensated cirrhosis (57 cases), compensated cirrhosis (27 cases), acute diffuse parenchymal degeneration including "hepatitis" due to chemical or bacterial toxins, and so-called catarrhal jaundice (19 cases), chronic cholecystitis (27 cases), obstructive cholelithiasis (8 cases), hepatic neoplasm (17 cases), obstructive neoplasm (4 cases), chronic congestive heart failure (17 cases), and occasional cases of amyloidosis, fatty degeneration, tuberculous hepatitis, hemachromatosis, and syphilitic hepatitis. Liver disease was excluded in 38 cases. In certain groups data are insufficient to permit evaluation of the results of tests, but the findings have been tabulated because of their bearing on the interpretation of results in the larger groups.

In this investigation the Takata-Ara reaction was done by the modified technique described by Heath and King,[7] serum albumin and globulin fractionation by Howe's procedure,[8] using the macro-Kjeldahl method for nitrogen determination, icterus index according to Meulengracht,[11] and the total and esterified cholesterol by means of differences in rate of the Liebermann-Burchard reaction as described by Reinhold.[12] Our studies of serum cholesterol fractionation, however, are not complete, and results are reported only in cirrhosis. The qualitative van den Bergh reaction has been utilized solely

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as a test for bilirubinemia and findings are not presented. Galactose tolerance tests were done at times but failed to give sufficient information to warrant further study.

The Takata-Ara reaction as a test for hepatic dysfunction has been studied intensively during recent years. Widespread interest has been created by the somewhat spectacular claim of specificity in the diagnosis of cirrhosis. Staub and Jezler first described the use of the test in diagnosis of liver disease, Hafström's[6] monograph includes a review of the literature, and Kirk's[9] paper summarizes recent work.

The occurrence of the various types of the Takata-Ara reaction in cirrhosis as shown in Table I agrees closely with similar percentages calculated from the data of Heath and King. However, it is clear that reactions grouped as "suspicious" (questionable flocculent precipitate) had little diagnostic

TABLE I

TAKATA-ARA REACTION

	STRONGLY POSITIVE	POSITIVE	WEAKLY POSITIVE	SUSPICIOUS	NEGATIVE
	NUMBER OF PATIENTS				
Cirrhosis	24	33	4	2	9
Other parenchymatous liver disease	5	10	4	3	10
Parenchymatous liver disease excluded	2	6	1	3	34
Total	31	49	9	8	53
Per cent having cirrhosis	78	67	44	25	17

significance, and that the accuracy of the test has been improved by considering these questionable reactions negative (as has been done in Table II). It should be noted that various authors differ in defining positive reactions and that results are not strictly comparable. We believe, however, that the technique of Heath and King employed by us has a higher precision in diagnosis of cirrhosis than that described by Crane,[4] since at times in the presence of cirrhosis, precipitates were obtained only in small amounts in either or both dilutions of 1:32 and 1:64, reactions that are negative by the criteria of Crane.

Positive Takata-Ara reactions were obtained in 45 of 49 patients having decompensated cirrhosis, and in 16 of 23 having cirrhosis without ascites. Positive tests were encountered also in other conditions associated with diffuse involvement of the liver: neoplasm, acute parenchymal degeneration, amyloidosis, fatty degeneration, and cholelithiasis of long duration. The reaction was positive in 3 of 14 cases of chronic congestive heart failure, and in 4 (subacute nephritis 2, pulmonary tuberculosis, lung abscess) in which liver disease was suspected and subsequently excluded.*

Change from a positive to a negative Takata-Ara reaction may occur with regression of the causative disease. This has been observed to coincide with or follow clinical improvement, not only in hepatitis and similar acute conditions but also in patients with unmistakable evidence of cirrhosis.

*This series includes only tests done because of a clinical suspicion of hepatic disease. The Takata-Ara reaction may be positive in a variety of extrahepatic diseases associated with serum protein changes, notably lobar pneumonia, tuberculosis, hyperthyroidism, multiple myeloma, subacute and chronic nephritis, and nephrosis (see Bowman and Bray[22]).

TABLE II

SUMMARIZED COMPARISON OF TESTS

DIAGNOSIS		ICTERUS INDEX		BROMSULFALEIN RETENTION PER CENT			TAKATA-ARA			SERUM ALBUMIN PER CENT		SERUM GLOBULIN PER CENT		ALBUMIN-GLOBULIN RATIO	
		3-9	10-220	0-5.9	6-9.9	10-100	NEG.	WEAK POS.	POS.	1.5-3.8	3.9-5.2	1.7-3.1	3.2-5.8	0.27-1.3	1.3-2.2
		NUMBER OF CASES													
Cirrhosis with ascites	Necr.	1	15	–	–	8	2	–	16	12	–	1	11	11	1
	Clin.	3	24	2*	–	11	2	2	27	23	1	9	15	23	1
Cirrhosis without ascites	Necr.	2	5	1	–	1	3	–	4	1	1	1	1	1	1
	Clin.	5	6	1	2	4	4*	2	10	7	1	4	4	5	3
Hemachromatosis	Biopsy	1	–	–	–	1	–	1	–	–	–	–	–	–	–
Neoplasm (obstructive)	Necr.	–	3	–	–	–	1	–	–	1	–	1	–	1	–
	Clin.	–	1	–	–	–	1	–	–	–	–	–	–	–	–
Neoplasm (parenchymatous)	Necr.	3	6	2	–	2	3	2	2	1	1	2	–	1	1
	Clin.	2	6	1	1	2	2	–	3	2	–	1	1	2	–
Cholecystitis	Necr.	–	2	1	–	1	–	–	–	–	–	–	–	–	–
	Clin.	6	8	11	6	7	5	–	–	1	3	3	1	1	3
Choledocholithiasis	Necr.	–	3	–	–	–	1	–	–	–	–	–	–	–	–
	Clin.	–	5	–	1	3	–	–	2	1	–	–	1	1	–
Acute diffuse parenchymal degeneration	Necr.	–	2	–	–	–	–	–	1	1	–	–	1	1	–
	Clin.	–	17	–	1	4	6	–	3	3	–	1	2	3	–
Fatty degeneration	Necr.	–	2	1	–	–	1	–	2	1	–	–	1	1	–
	Clin.	–	–	–	–	2	–	–	–	–	1	1	–	–	1
Tuberculous hepatitis	Necr.	1	1	–	–	–	–	–	1	2	–	–	2	2	–
Syphilitic hepatitis	Biopsy	1	–	–	–	1	–	–	–	–	–	–	–	–	–
	Clin.	–	1	–	–	1	–	–	1	1	–	–	1	1	–
Amyloidosis	Necr.	–	–	–	–	–	1	–	1	1	–	–	1	1	–
	Clin.	–	–	–	–	1	–	1	–	–	–	–	–	–	–
Congestive heart failure	Necr.	–	2	–	–	2	2	–	–	1	–	–	1	1	–
	Clin.	1	3	1	2	4	9	–	3	3	2	4	1	2	3
Liver disease excluded	Necr.	2	–	3	–	–	1	–	2	2	–	–	2	2	–
	Clin.	7	2	10	1	2	17	1	1	7	4	7	4	6	5

*One subsequently positive.

Since various authors have shown that the Takata-Ara reaction is closely related to abnormalities of the serum proteins, it seemed desirable to ascertain whether fractionation of the proteins might not prove to be a more reliable test. The prevalence of abnormally altered concentrations of albumin and globulin in the presence of diffuse involvement of the liver as shown in Table II is in accord with other reports.[1] In 58 of 72 patients with liver disease (of whom 46 had cirrhosis) albumin-globulin ratios were less than 1.3. Sixty-two of this number had less than the minimum normal concentration of serum albumin, while 44 showed a rise in globulin above the upper limit of normal. Loss of albumin was usually compensated wholly or in part by increased globulin, so that serum total protein was infrequently abnormal. However, in the presence of malignancy or other wasting disease, low total proteins were characteristic. Here change was chiefly in the albumin fraction.

Almost every patient with decompensated cirrhosis showed abnormally low ratio of albumin to globulin. Likewise, only one patient showed an albumin concentration within the normal limits. Elevation of serum globulin seemed to be more prevalent in the more severe and terminal cases of cirrhosis. In general, alteration in serum proteins occurred less frequently in compensated cirrhosis. The incidence of abnormal albumin-globulin ratio in all stages of cirrhosis was almost identical with that of positive Takata-Ara reactions. However, in 3 patients abnormally low ratios were associated with negative Takata-Ara reactions, while 6 with normal ratios gave positive Takata-Ara reactions. Again, 6 patients with inversion of the albumin-globulin ratio caused by renal disease gave negative Takata-Ara tests, although positive tests have been observed in others with renal disease. Similar findings have been reported by other workers.[15, 20-22] It is apparent that factors other than the ratio of albumin to globulin influence the characteristic flocculation of the Takata-Ara reaction.

Serum cholesterol fractionation has been used in the investigation of liver disease since the demonstration by Thannhauser and Schaber[19] that esterified cholesterol decreased to low values when widespread injury to the liver occurred. Changes occurring in cirrhosis have been investigated by Epstein and Greenspan[5] and Adler and Lemmel.[2] In the present study total and esterified cholesterol of serum was determined in one or more specimens from 48 patients with cirrhosis.

While the concentration of total serum cholesterol with a single exception remained within the limits of normal (found to be 100 to 350 mg. per 100 c.c.), low normal values appeared with increased frequency. Concentrations of cholesterol combined as esters were often below normal in the presence of icterus (Table III). Cholesterol ester values in patients with cirrhosis and jaundice who died were compared with values obtained in those who were similarly jaundiced (icterus index over 15) but recovered. No significant difference was found.

The correlation of these newer tests with the widely used bromsulfalein and icterus index determinations is important. Following the introduction of bromsulfalein by Rosenthal and White,[14] various workers[3] demonstrated that retention occurred frequently in the absence of jaundice and that the

*One subsequently positive.

test was superior to most for the detection of cirrhosis. Originally 2 mg. of dye per kilogram of body weight was recommended, but later Greene advocated increasing the dosage of dye to 5 mg. The larger amount has been used in these studies. It appears that the proportion of the injected dye that is retained by normal individuals when 5 mg. per kilogram are administered has not been established with certainty, for various authors have regarded values ranging from 4 to 10 per cent retention at thirty minutes as normal. Although Soffer[17] states that at the end of one-half hour never more than a trace of the dye has been found to be present in normal individuals, in a subsequent publication[18] he regards 10 per cent retention at thirty minutes as the dividing limit. To clarify this detail, retention of the dye has been measured in 8 normal individuals. Between 0 and 6 per cent of the administered bromsulfalein remained in blood serum taken thirty minutes after the injection. One individual showed no retention, one showed 2 per cent, three showed 3 per cent, and one each 4, 5, and 6 per cent. These results suggest that the average amount of dye normally retained is between 3 and 4 per cent.

TABLE III

RELATIONSHIP OF TOTAL AND ESTERIFIED SERUM CHOLESTEROL TO ICTERUS INDEX IN CIRRHOSIS

		TOTAL CHOLESTEROL			ESTERIFIED CHOLESTEROL		
		MG. PER 100 C.C. SERUM					
		95-150	150-250	250-350	8-50	50-70	70-160
		NUMBER OF PATIENTS					
Icterus index	Less than 15	7	8	3	2	6	10
	15 and over	12	23	7	15	8	19

Confirmation was given by tests done on 16 schizophrenic patients without apparent somatic disease. Three patients showed no retention, three showed 3 and 4 per cent respectively, 4 showed 5 per cent, and one each 6, 7, and 8 per cent. Because of their occasional occurrence in normal individuals, values between 6 and 9.9 per cent have been grouped separately in Table II. However, we believe that nearly all figures within this range are indicative of impaired excretory function.

In cirrhosis with ascites, the bromsulfalein test indicated distinctly abnormal liver function in 19 of 21 patients. One of the two negative tests was repeated several weeks later and definite retention found. In compensated cirrhosis, 6 tests were positive, two between 6 and 10 per cent, and therefore suspicious, and two were negative. In the latter two, the Takata-Ara reaction was positive. On the other hand, dye retention occurred in 5 patients with cirrhosis in whom the Takata-Ara reaction was negative.

Bromsulfalein injection detected hepatic insufficiency resulting from neoplastic metastasis to the liver in 3 patients without jaundice, but failed in 3 with icterus indices of 6, 8, and 14. Retention occurred with noteworthy frequency in cholecystitis and in chronic congestive heart failure. Occasionally the bromsulfalein test was positive in the absence of liver disease. Of 17 patients without demonstrable disease of the liver, 3 showed retention exceeding 6 per cent, the figures being 9, 12, and 14 per cent.

The use of bromsulfalein was restricted to patients with little or no jaundice. Marked retention has been found to occur invariably in the presence of jaundice, and the difficulty of determining accurately the degree of retention in highly icteric sera impairs the value of the test for comparative purposes. The choleretic action of bromsulfalein affords another objection to its use in patients with deep jaundice of uncertain etiology.

The estimation of icterus index is a helpful procedure, for abnormal concentration of bile pigment in the blood may exist in the absence of clinical jaundice. Although Meulengracht, who introduced the test, regarded 10 as the upper limit of normal, 4 to 6 is commonly, yet erroneously, accepted as the normal range. In this laboratory, values as high as 9 are encountered in healthy individuals. Values above 9 occurred in more than 90 per cent of patients with decompensated cirrhosis, but in only 60 per cent of those without ascites. Ten patients with cirrhosis had normal icterus indices and positive Takata-Ara reactions; frequently the latter was strongly positive with only minor elevation of the icterus index. The marked elevation of the icterus index encountered at times in congestive heart failure is noteworthy. Two patients without demonstrable disease of the liver had icterus indices of 10 and 11. We continue to find a relationship between icterus index values and type of qualitative van den Bergh reaction, in agreement with the observations described in an earlier report.[16]

DISCUSSION

Our data indicate that the tests investigated are sufficiently accurate to make them effective supplements to clinical examination for diagnosis of liver disease. The results demonstrate anew that none of the tests appraised can be relied upon to give conclusive evidence of minimal lesions of the liver and therefore that several tests should be employed where doubt exists.

Frequently the results of these tests have merely substantiated diagnoses clinically obvious. Nevertheless, their special value has been demonstrated on several occasions when clinical findings were inconclusive. This has been true particularly when bromsulfalein retention or the Takata-Ara test have given the only tangible evidence of liver involvement before development of ascites and other manifestations of the advanced stage of the disease. Information secured by the application of these tests has been useful also in differentiating hepatic from other abdominal tumors, and in indicating the cause of hematemesis to be varices due to cirrhosis rather than ulcer.

The bromsulfalein test was somewhat more sensitive and accurate than the others studied. Although a slightly different procedure was used, our conclusions in this respect agree with those of Magath.[10] It can be used most effectively when jaundice is absent or slight; with the serum bilirubin concentration markedly elevated, the bromsulfalein test is superfluous and unsatisfactory. Rarely failing to detect liver damage when this was demonstrated by other methods, the dye test was frequently the only one to reveal hepatic disease. Retention in extrahepatic diseases noted by Robertson, Swalm, and Konzelmann[13] occasionally has been encountered. It is questionable whether involvement of the liver sufficient to cause dye retention could

not have existed in these patients despite clinical evidence to the contrary. If 10 per cent retention at thirty minutes is regarded as the limit of normal when 5 mg. per kilogram are injected, the accuracy of the test is diminished somewhat. Undoubtedly, the use of this limit explains to some extent the unfavorable conclusions of Soffer and Paulson[18] concerning the bromsulfalein test as compared with the bilirubin retention test. As pointed out, only 4 of 24 controls in our series showed retention in the 6 to 9.9 per cent range. We believe, therefore, that retention in the borderline region between 6 and 9.9 per cent should be regarded as a probable indication of liver insufficiency.

The Takata-Ara reaction, which is easily performed, may be advantageously used either as a preliminary or as a confirmatory test. In the detection of early cirrhosis, it is somewhat less sensitive than the dye test. Positive in most cases of advanced cirrhosis, it is often positive as well in other conditions extensively involving the liver, and cannot be relied upon to differentiate these conditions from cirrhosis. Crane's[4] conclusion that the chief merit of the test lay in its ability to distinguish cirrhosis from tumors was not substantiated by our findings, for positive reactions were frequent (although less common than in cirrhosis) in neoplastic disease of the liver. Although lacking specificity for hepatic disease, positive reactions were misleading only in a tuberculous patient who was thought to have an incidental cirrhosis; necropsy revealed no lesion of the liver. Two cases of subacute nephritis with anasarca considered to be possible cirrhosis gave positive Takata-Ara reactions, but the correct diagnosis was established without difficulty. A common and sometimes perplexing problem is that of differentiating the ascites of cirrhosis from that of congestive heart failure. Here, where bromsulfalein retention is, as a rule, marked and may be interpreted erroneously as indicating cirrhosis, the Takata-Ara test is less often misleading; a positive reaction is suggestive of cirrhosis.

The frequency with which serum albumin was lowered and globulin increased in diseases of the liver is especially worthy of note. Although the Takata-Ara test seemed a more dependable guide to the recognition of liver lesions, nevertheless diminution or inversion of the albumin-globulin ratio should be recognized as a common manifestation of diffuse liver disease.

The icterus index was infrequently elevated in the absence of liver injury. Chronic liver disease, however, was often revealed by bromsulfalein retention, a positive Takata-Ara reaction, and inversion of the albumin-globulin ratio with the icterus index normal.

The concentration of total cholesterol in serum was not markedly altered in cirrhosis, although low values were common. Esterified cholesterol was more frequently diminished both in concentration and in percentage of total cholesterol. Single determinations were of no value in distinguishing cirrhosis with transient superimposed "hepatitis" from terminal hepatic failure.

CONCLUSIONS AND SUMMARY

Application of five tests to the study of liver function in 228 patients under routine conditions in a large general hospital has demonstrated their value as a supplement to clinical methods in the diagnosis of cirrhosis and

other diseases of the liver. For the detection of liver disease before it had reached an advanced stage, the bromsulfalein retention test, the Takata-Ara reaction, and fractionation of serum proteins, were more useful than icterus index and cholesterol or cholesterol ester determinations.

Discrimination should be exercised in the selection of tests; when doubt exists it is desirable to employ several tests in conjunction with one another.

The rate of excretion of bromsulfalein by normal individuals has been reexamined.

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ANALYSIS OF FLUID FROM A CHYLOUS MESENTERIC CYST*

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CHYLOUS cysts of the human mesentery are not of common occurrence, and chemical analyses of their contents are encountered in the literature only rarely. Since the cause of these cysts is still uncertain, it seemed desirable to report the following work:

REPORT OF CASE[9]

A forty-eight-year-old woman was admitted to the Surgical Service of St. Margaret Memorial Hospital, complaining of a gradual increase in the size of her abdomen, with accompanying discomfort, over a period of about four years. Palpation revealed a tumor in the left upper quadrant which was freely movable from side to side. It was painless, cystic in feel, and gave a flat note on percussion. Exploratory laparotomy disclosed a tumor mass 29 cm. in diameter. The cyst originated in the mesentery, close to its spinal attachment, at about the level of the first lumbar vertebra. Since a loop of small intestine was densely incorporated in the wall of the cyst, and the aorta and vena cava seemed to be intimately connected with its site of attachment, marsupialization was resorted to. About 1,000 c.c. of fluid,

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presently to be described, was aspirated from the cyst through a trocar and cannula. The trocar opening was enlarged, the cyst drawn out of the abdomen through the incision, and its walls sutured to the abdominal wall. The incision was then closed above and below the cyst, and the latter packed with gauze. The patient made a satisfactory recovery; the sinus formed by the cyst cavity eventually becoming completely obliterated.

On April 21, 1936, the sample of fluid aspirated at operation was submitted to the laboratory for analysis. It consisted of 750 c.c. of a thick, creamy fluid, out of which, on standing, settled a white, tenacious caseous sludge. In order to avoid interference with analytical procedures and to observe the purported resistance of chlyous fluids to putrefaction, no preservative was added. The flask, which had hitherto been provided with a cotton plug, was stoppered with a clean cork (to prevent evaporation), and kept, when not in use, in an ice refrigerator.

ANALYTICAL METHODS

Before sampling, the contents of the flask were shaken vigorously for about two minutes, and the bottom of the flask examined for adherent sludge, to make certain that all solid matter was thoroughly and uniformly suspended. The sedimentation rate of the sludge was slow; consequently, a thorough agitation when the flask was removed from the refrigerator, and a briefer shaking prior to the pipetting of each sample, insured homogeneity of the samples.

Specific Gravity was determined by weighing, in a tared flask, a pipetted 5 c.c. sample of the fluid, and comparing its weight with that of 5 c.c. of water at the same temperature.

Total Solids.—25 c.c. portions of the fluid were evaporated to a paste on a water-bath in tared dishes, and dried to a weight constant within 1 mg. under a vacuum of 60 to 120 mm. of mercury.

Ash.—The dried residues from the above determination were transferred, with the aid of a little water, to tared crucibles, and ignited to constant weight.

Total Nitrogen.—5 c.c. of the fluid were diluted to 100 c.c., and nitrogen determined on 1 c.c. aliquots of this solution, using Van Slyke's manometric hypobromite method.[4]

Nonprotein Nitrogen.—5 c.c. samples of cyst fluid were precipitated by Haden's modification[5] of the tungstic acid method of Folin and Wu, and the nitrogen in aliquots of 1 to 5 c.c. of the filtrate, determined by the method used for total nitrogen.

Protein Nitrogen was calculated as the difference between total and nonprotein nitrogen.

Protein was calculated from protein nitrogen, using the customary factor of 6.25.

Lipids.—Total fat, free fatty acids, soaps, and neutral fat, were determined on 5 c.c. portions of the fluid, using Fowweather's modification of Saxon's method for lipids in stools.[6] Because excessive emulsification occurred when the fluid was extracted by shaking with alcohol and ether, a microconstant extraction apparatus was adapted for this purpose.

Calcium.—5 c.c. of cyst fluid were digested with a sulphuric-nitric acid mixture until clear, and made up to 100 c.c. Aliquots of this mixture, neutralized to phenol red with dilute ammonia, were analyzed for calcium by the micromethod of Tisdall and Cramer, modified by Stanford and Wheatley.[7]

RESULTS

Results of the present analysis are listed in Table I, which includes for comparison, analytical figures for chylous cysts, chylous effusion, and chyle, taken from the literature.

DISCUSSION

Analytical procedures have not been specialized for chyle as for blood and urine. It was consequently necessary to adapt standard clinical laboratory methods to the purpose of this examination. Although these may not be thought to yield with chylous fluids a degree of accuracy as high as with

TABLE I

ANALYSES OF CHYLOUS FLUIDS

	CHYLOUS CYSTS				CHYLOUS EFFUSION	CHYLE	
	AUTHOR'S CASE	SCHUMM[8]	ZDAREK[12]	MORLOT AND JENNESSEAUX[2]	BUCHTALA[1]	PANZER[3]	SOLLMAN[10]
Volume c.c.	750 (1000)	1500	70	700	-	-	-
Specific Gravity	1.0120	0.988	-	1.004	1.017	-	-
			Per Cent				
Total Solids	12.45	39.8	10.58	13.18	8.66	9.71	7.10
Ash	1.85	0.92	0.84	0.95	0.95	0.81	0.60
Calcium	0.486	0.15	-	-	0.09	-	-
Total Nitrogen	0.786	-	-	-	0.750	-	-
Nonprotein Nitrogen	1.60	-	-	-	-	-	-
Protein Nitrogen	0.608	-	-	-	-	-	-
Protein	3.84	1.97	7.19	8.51	4.69	2.16	1.85
Total Fat	4.50	35.8	2.70	4.66	1.147	6.59	1.93
Free Fatty Acids	1.30	-	-	-	-	-	-
Soaps	1.50	0.43	-	-	-	-	-
Neutral Fat	1.70	-	-	-	-	-	-

the materials for which they were intended, it is nevertheless felt that they are sufficiently exact to permit comparison between the composition of the chylous fluid here described, and the results found by other workers.

Since several days had elapsed between the collection of the fluid and the beginning of the analysis, determination of sugars was not attempted. It is highly probable that any sugar originally present would have been more or less completely destroyed by glycolysis.

As may be seen in Table I, there is a wide range of variation in the figures reported by the various workers who have analyzed chylous fluids. This may be due partly to differences in analytical methods, but can be satisfactorily explained by the innate variability in composition of chylous fluids, and the effect of various factors of dehydration, differential absorption, etc., acting on a stagnant fluid. With a few exceptions, noted presently, the present figures agree well with those previously reported.

In determining protein we have assumed that the nitrogen precipitated by tungstic acid under the conditions of the customary Folin-Wu blood precipitation method and protein nitrogen, were identical. Sollman[10] and Buchtala[1] calculated protein from total nitrogen alone; while the other workers cited here weighed coagulable matter and reported it as protein. Each of these methods is open to error when dealing with proteins of unknown properties. The relatively large amount of nonprotein nitrogen found suggests that a considerable amount of proteinlike substance escaped precipitation.

The author's figure for lipids lies well within the range of values previously reported. Although we lack sufficient data for comparison, the amount of soaps seems high. Because of the high calcium content of the fluid, one assumes the soap fraction to consist mainly of calcium soaps.

The feature which distinguishes the composition of the fluid under discussion from that of the other listed fluids is its high mineral content, and the high proportion of the latter represented by calcium. Of the 486 mg. per cent of calcium found, only 109 mg. would be necessary to combine with the fatty acids of the soap fraction, assuming the latter to be calcium palmitate. It seems possible that the high mineral content may, perhaps, indicate a beginning calcification of the contents of the cyst.

The ash, protein, and total lipids found comprise 82 per cent of the total solids, while, if the nitrogenous bodies not precipitated by tungstic acid (nonprotein nitrogen fraction) were assumed to be proteinlike, 90 per cent of the solids would be accounted for.

BACTERIOSTATIC ACTION OF THE FLUID

Wells[11] points out that chylous effusions possess marked bacteriostatic properties, and the phenomenon is also mentioned by Morlot and Jennesseaux.[2] The author's experience corroborates these observations. Although no further effort was made to keep the fluid studied in this work sterile than, in general, to store it in a refrigerator when not in use, it remained for over two months without showing gross evidence of putrefaction or fermentation. Cultures taken from it on May 22 showed a few colonies of gram-negative bacilli (saprophytes?). On June 25 a faint odor of putrefaction was first noticed.

That a fluid of relatively high protein content could remain so long unputrefied, in spite of ample opportunity for contamination, and in the presence of a proved bacterial contamination, cannot be explained by the refrigeration to which it had been subjected. The temperature in the refrigerator averaged 10° C., and there were occasions when the flask containing the fluid was, by oversight, allowed to remain from eighteen to twenty-four hours in a room whose temperature varied from 25° to 30° C. It seems evident that the chylous fluid we studied possessed bacteriostatic activity which, toward the end of a two months' period, gradually disappeared; either by chemical change, or by being overwhelmed by successive bacterial contaminations.

SUMMARY AND CONCLUSIONS

1. The results of a chemical analysis of fluid from a chylous mesenteric cyst are reported.

2. With the exception of high values for ash and calcium, these results agree with previously reported analyses of chylous cyst contents and other chylous fluids.

3. It is suggested that the high content of inorganic salts, including calcium, may possibly be due to beginning calcification of the cyst.

4. The observation of others, that chylous fluids possess bacteriostatic activity, is confirmed for the fluid studied in this work.

The author wishes to acknowledge the cooperation of Dr. M. A. Slocum, of the Surgical Department of St. Margaret Memorial Hospital, for placing the fluid at his disposal for analysis, and for furnishing the surgical and clinical data relating to the patient from whom it was obtained.

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THE EFFECTIVENESS OF THERAPEUTIC MEASURES AFTER POISONING WITH SUBLETHAL AND LETHAL DOSAGES OF BARBITAL IN THE RABBIT*

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THE efficacy of analeptics in the presence of poisoning from hypnotics or narcotics bears an inverse relation to the duration of action and depths of hypnosis of the depressant agent, according to Maloney, Fitch, and Tatum (1931), Maloney and Tatum (1932), and Swanson (1932). Resuscitation measures are thus symptomatically more effective against the short acting hypnotics of the pentobarbital, amytal, or avertin type (Barlow, 1935), than against barbital per se (Maloney and Tatum, 1932; Linegar, Dille, and Koppanyi, 1935). Gros and Hofmann (1936), on the other hand, have shown that combined therapy with optimal dosages of metrazol (pentamethylene tetrazol) and ephedrine reduces the mortality of an 80 per cent lethal dosage of barbital sodium in rats to zero. The significantly lower efficiency of analeptics in general, under conditions of barbital or phenobarbital poisoning, as compared with other hypnotics of the same series, is due largely to the greater stability of the long acting preparations. These compounds are firmly fixed in the tissues and are excreted largely unchanged over a period of seven to nine days.

The generally unfavorable prognosis under clinical conditions of poisoning with lethal dosages of barbital under present methods of therapy suggested the need of further study of the problem.

METHODS

Adult albino rabbits, weighing 2.5 to 3.5 kg. were used in all experiments. The diet fed for at least three weeks before medication of any animal included alfalfa, hay, oats, and water ad lib. Fresh clover and lettuce were added twice weekly.

The hypnotic was administered intraperitoneally as a 10 per cent aqueous solution of barbital sodium (Merck and Company) in each dosage series tested.

The efficacy of each agent and of combinations of the more satisfactory antidotal measures was tested under conditions of minimally hypnotic dosages (150 mg. per kg.), maximally hypnotic and exceptionally lethal dosages (300 mg. per kg.), a usually lethal (85 per cent) dosage of 450 mg. per kg., and after medication with not less than two absolute lethal dosages (1,000 mg. per kg.) of barbital sodium.

The therapeutic agents or measures tested included "coramine" (N.N. diethyl nicotinamide), ephedrine, metrazol (pentamethylene tetrazol), piero-

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toxin, caffeine sodio-benzoate, the intravenous administration of dextrose, and combinations of the more effective agents of this group (Barlow, 1935).

Treatment was instituted in each series of experiments at the time hypnosis became maximal or respiratory depression became marked, i.e., one hour after medication with minimal dosages, and within fifteen to twenty minutes after maximal dosages of the hypnotic.

Therapy during the acute stages of poisoning was invariably by the intravenous route. In later stages of treatment intramuscular routes of medication were used. All animals, with the exception of those in the lowest dosage group, were treated after medication with the hypnotic at intervals as required by their condition up to forty-eight hours. Exceptionally in the lethal dosage groups, additional treatment up to seventy-two hours was required.

RESULTS

The antidotal values of different dosages of the several analeptics against the depression of minimal hypnotic dosages (150 mg. per kg.) of barbital sodium were determined on groups of 7 to 15 rabbits each. The control reactions of individual animals of each group to barbital alone were tested at least twice at ten-day intervals before the antidotal effects of any analeptic were studied. The observations made during the course of recovery from hypnosis included the time of spontaneous lifting of the head, sitting, and complete recovery. The final observation is most dependent on the experience of the observer, in that reliable end points are only obtainable by careful observations of spontaneous activity, equilibrium, etc., when the animals are free to move about in the absence of noise or other disturbing factors. All data were reduced to medians. Only the reactions following treatment (over a period of two to eight hours) with optimal dosages of each medication sequence will be discussed.

Maximal hypnosis following the intraperitoneal injection of barbital sodium in a dosage of 150 mg. per kg. occurred within 0.77 to 1.15 hours. Individual animals were relatively standard in their reactions. The initial stage of recovery in untreated controls (spontaneous lifting of the head) occurred within a range of 2 to 3.25 hours with a median of 2.3 hours after medication.

The evaluation of the several therapeutic measures differs somewhat, depending upon the time at which the different stages of recovery occur. Thus the order of efficiency from greatest to least as to the "awakening" effects is as follows: metrazol > picrotoxin and ephedrine > picrotoxin > ephedrine > coramine > caffeine > dextrose infusion. On the basis of the degree of shortening of the normal sitting or hopping time, the observed order of efficiency was as follows: metrazol > picrotoxin and ephedrine > ephedrine > picrotoxin > coramine > caffeine > dextrose. On the basis of the degree of shortening of the normal time for complete recovery from the hypnotic, the observed order was: metrazol and ephedrine, picrotoxin and ephedrine, picrotoxin, metrazol, ephedrine, dextrose infusion, coramine, caffeine. The latter two agents had no apparent shortening action on the normal complete recovery time.

Optimal antagonistic dosages of the several analeptics (in milligrams per kilogram) administered in four to six divided doses after the development of maximal sedative reactions to barbital are as follows: metrazol (250), picrotoxin (5), ephedrine (15), caffeine (150 to 250), coramine (450 to 750), dextrose (75 to 100 c.c. per kg. of a 5 per cent solution by vein), picrotoxin (5), and ephedrine (10).

Maloney and Tatum (1932) have reported that a dosage of 300 mg. of barbital sodium intraperitoneally killed 83.4 per cent of a series of 6 rabbits. The lethal effects of such dosages were reduced to 33 per cent under treatment with picrotoxin. Results of the present study indicate, due presumably to racial variability, that a dosage of 300 mg. of barbital per kg. killed only 2 of a series of 11 albino rabbits. This lethal figure was reduced to zero in a total of 36 rabbits by careful therapy at intervals of groups of 6 to 8 animals, with optimal dosages of metrazol (400 to 500 mg. per kg.), metrazol and ephedrine (15 mg. per kg.), picrotoxin (15 mg. per kg.), or dextrose (75 to 100 c.c. per kg. of a 5 per cent solution) intravenously. On the other hand, medication with effective dosages of caffeine appeared to be actually detrimental, i.e., the mortality under such therapy exceeded that of the control series.

The usually accepted minimum lethal dosage, i.e., that which kills 50 per cent of a series, was considered quite unsatisfactory from the standpoint of making an accurate evaluation of antidotal measures, in that under conditions of treatment, a 100 per cent survival would represent only a 50 per cent efficiency for the therapeutic measures under test.

The difficulties involved as to the interpretation of results of various antidotal measures after intraperitoneal injections of a 50 per cent lethal dosage of barbital sodium are illustrated by the variability of this figure in the hands of different observers. Using mixed breeds of rabbits, Fitch and McCandless reported that a dosage of 250 mg. of barbital sodium intraperitoneally killed 60 per cent of a series. Maloney and Tatum (1932) observed that 300 mg. per kg. killed 83.4 per cent. Jones and Lynn (1935) reported that 375 mg. per kg. killed only 50 per cent of a small series of animals, but a dosage of 385 mg. per kg. was 100 per cent lethal.

It was deemed necessary, therefore, to determine the toxicity of barbital sodium on intraperitoneal administration as a basis for a proper evaluation of the analeptic measures under study. The dosages tested ranged from 0.25 to 1.0 gm. per kg. body weight. All dosage groups included a total of 70 animals. None of the groups had received any of the barbituric acid derivatives previous to this test. These data indicate that 0.25 gm. per kg. killed only one of ten animals, 0.3 gm. per kg. killed 2 of 11 animals, and 0.4 gm. killed 4 of 11 animals. The 60 per cent M.L.D. is 0.425 gm. per kg. The absolute lethal dosage by this route exceeds 0.45 gm. per kg. (killed 90 per cent of a series of 20 animals).

450 mg. per kg.—The first group of animals in the treated series of experiments was medicated with metrazol over a period of only twelve hours. All animals were nearly normal twelve hours after the beginning of treatment. Due to lack of further treatment, relapses occurred and all animals were dead on return to the laboratory at the twenty-second hour after barbital medication.

TABLE I

BARBITAL SODIUM (10% SOLUTION) 450 MG./KG. INTRAPERITONEALLY

RABBITS PER SERIES	TREATMENT	TOTAL DOSAGE MG. OR C.C./KG.	TREATMENT TIME—HOURS	MEDIAN RECOVERY TIME—HOURS	RECOVERY PER CENT OF SERIES	TIME OF DEATH HOURS	DEATHS PER CENT OF SERIES	REMARKS
15	Metrazol Pierotoxin Ephedrine Glucose 5%	300 10 7½ 75 c.c.	48	60	93.34	36	6.66	Single death was due to over-treatment with pierotoxin.
11	Pierotoxin Metrazol	15 500	48	72	90.81	47	9.09	
11	Pierotoxin Glucose 10%	36 75-100 c.c.	48-60 48	72	90.81	39	9.09	
17	Metrazol	1000-1400	28	72	83.3	64.5	16.6	
10	Metrazol Glucose 5%	600-800 100 c.c.	48-50 30	72-84	80	56 (39-59½)	20	
11	Pierotoxin	16-20	60	72	71.8	40 (36-43)	28.2	
16	Glucose 5%*	100 c.c.	36	72	50	15 (8-46)	50	
5	Coramine	750	During survival	--	0	13½ (5¼-26)	100	Significant secondary depression.
20	Controls	---	----	65.85 (60-120)	10	11½ (3½-12)	90	

*Total volume of sugar solution was injected by vein in 4 to 6 divided doses.

The efficacy of various therapeutic measures, following the intraperitoneal injection of the 90 per cent lethal dosage of barbital sodium is illustrated in Table I. It was possible by careful symptomatic control of respiration and indirect control of the circulation, by means of a combined therapy with picrotoxin, metrazol, ephedrine, and 5 per cent glucose by vein, to save 93.3 per cent of a series of 15 animals; while medication with picrotoxin and metrazol, or picrotoxin and 5 per cent glucose by vein, saved similar percentages (90.8) of groups of 11 animals.

The intravenous administration of 5 per cent dextrose at intervals over a period of forty hours was of some antidotal value, in that under such treatment, more than twice as many survivals occurred as were noted in the control series. The effectiveness of fluids was further demonstrated when this procedure was combined with coramine. Such combined therapy saved 50 per cent of the treated animals, although all animals of a second group treated with coramine alone died. Effective dosages of coramine were prone to produce a significant secondary depression, generally accompanied by pulmonary edema.

The mechanism of action of the intravenous glucose in decreasing the lethal effects of barbital is explained only in part. Unpublished data on dogs indicate that 10 per cent glucose by vein results in a small but significant acceleration of the normal urinary excretion rate of barbital. The diuretic effects of the injected fluid on rabbits were marked. Koppanyi, Murphy, and Krop (1933) observed no acceleration of the barbital excretion rate in dogs when treated with 10 per cent glucose by vein. Johnson, Luckhardt, and Lighthill (1930) reported that the duration of barbital hypnosis in dogs was reduced 50 per cent by intravenous glucose medication. The period required for complete recovery of barbitalized rabbits under the fluid therapy was not significantly altered, although the depth of hypnosis was significantly diminished. These data suggest that 5 per cent dextrose is superior to higher concentrations in the volume injected. The primary beneficial effects of isotonic glucose by vein are visualized as maintaining a positive water balance and the prevention of the development of acidosis as reported by Henze (1936), and Fischer and Salzer (1936).

1,000 mg. per kg.—The median time of death in a series of 7 rabbits medicated intraperitoneally with a dosage of one gram of barbital sodium per kilogram body weight, considered to be twice the absolute lethal dosage for rabbits, was thirty-eight minutes, with a range of twenty-five to sixty-nine minutes.

The order of efficiency from greatest to least of the most satisfactory analeptic measures tested, based on data obtained from the treatment of poisoning with smaller dosages of barbital, is illustrated in Table II.

All types of symptomatic treatment were effective in prolonging the survival period, in that death occurred only after eleven hours in the series treated with the least effective therapeutic sequence, as compared with a median of thirty-eight minutes in the control or untreated group. In other words, control of respiration and indirectly of the circulation with picrotoxin, metrazol, and ephedrine, together with fluids (5 per cent glucose) intravenously as required over a period of thirty-six to sixty hours, reduced the

TABLE II

BARBITAL SODIUM 1.0 GM./KG. INTRAPERITONEALLY

NUMBER OF RABBITS PER GROUP	SERIES	TREATMENT	TOTAL DOSAGE MG. OR C.C./KG.	NUMBER OF DOSES	TREATMENT TIME HOURS	RECOVERY PER CENT OF SERIES	TIME OF COMPLETE RECOVERY DAYS	DEATHS PER CENT OF SERIES	TIME OF DEATH HOURS		REMARKS
									MEDIAN	RANGE	
7	1	Controls	--	--	--	0.0	--	100.0	0.63	0.4-1.15	
6	2	Picrotoxin	39	11-16	48	83.3	4.5	16.7	39.5		
		Metrazol	1800-2200	16-22	48						
		Ephedrine	20	4	36						
		Glucose 5%	90-125 c.c.	4-6	36						
11	3	Picrotoxin	21	10-16	50	63.63	33.5	36.37	21	19-38	One animal died due to insufficient treatment
		Glucose 5%	130 c.c.	7							
11	4	Picrotoxin	25.37	18	48.60	54.55	5	45.45	20	12-2.20	
11	5	Picrotoxin	36	16	48	30	44	70	16.5	11-38.5	No treatment between 12th and 23rd hours; 5 out of 7 died during this time
		Metrazol	1,450	18	60						
		Ephedrine	25	4	36						
		Glucose 5%	100 c.c.	4	36						
11	6	Same as series 5	No treatment between 5th and 12th hours					81.82	7 out of 9 died between the 6th and 12th hours.		

mortality from 100 per cent to 16.7 per cent. The method of treatment used in the initial experiments of this dosage group was a duplication of the above therapeutic sequence, with the exception that no treatment was given between the fifth and twelfth hours due to the apparently excellent condition of the animals. The survival percentage of this initial series was only 18.8 per cent, i.e., seven of the nine fatalities occurred during this period because of insufficient medication. This picture is almost duplicated by discontinuance of therapy between the twelfth and twenty-third hours after the injection of the hypnotic.

DISCUSSION

Symptomatic control of the effects of barbital sodium poisoning in rabbits appears to be best accomplished by means of frequent medication with two central convulsants—picrotoxin and metrazol, the judicious administration of a vasopressor agent, ephedrine, and the administration of fluids intravenously preferably in the form of 5 per cent glucose. The efficacy of fluid in a corresponding volume of 10 per cent glucose or Ringer's solution was inferior to isotonic glucose. Medication should be continued at short intervals at the outset of the poisoning as judged by the symptoms present, and at longer intervals as the depth of hypnosis diminishes. In the presence of one or more absolute lethal dosages, treatment should be continued for a period of at least thirty-six hours for optimal results. The intervals of treatment depend on the condition of individual animals.

The administration of picrotoxin results in a submaximal stimulation of respiration, and reactions become maximal only after a latent period. The product in these respects is inferior to metrazol for emergency purposes. However, the duration of action of picrotoxin, which ranges from thirty to one hundred eighty minutes, depending on the depth of hypnosis, is superior to that of metrazol. On the other hand, picrotoxin is not wholly free of undesirable side actions, in that significant secondary depressant effects may develop after excessive dosages.

The respiratory stimulant effects of metrazol were definitely superior to those of other agents tested. Its duration of action may be as short as ten minutes in poisoning with massive dosages of pentobarbital, chloral, avertin, or barbital, but generally persists for twenty-five to forty-five minutes in lighter grades of poisoning. This product is best suited for emergency purposes under conditions of respiratory failure. On numerous occasions spontaneous respiratory movements have been initiated with metrazol intravenously in the presence of continued heart action, one or two minutes after cessation of respiration. An optimal sequence of agents for use in emergencies of respiratory depression or arrest would be a combination of both metrazol and picrotoxin. This sequence would result in a prompt and marked stimulation (ten to thirty minutes) of respiration by metrazol which in turn would be summated slightly and prolonged by the additional medication with picrotoxin. The optimal therapeutic level is best judged by maintenance of a good color (absence of anoxemia) and an abnormal reflex excitability. The effects of excessive dosages of either of these two analepties are readily controlled by pentobarbital by vein.

Ephedrine injected intravenously in maximal dosages of 2.5 to 5.0 mg. per kg. has a definite place in the antidotal sequence against hypnotic or narcotic poisoning. The optimal treatment interval with such dosages in rabbits appears to be between three and six hours. Larger dosages of ephedrine are prone to produce a heart block of greater or less degree and an embarrassment of respiration due to the development of pulmonary edema.

The administration of fluids by vein relieves the anuric effects of barbital, accelerates the urinary excretion of barbital slightly, maintains a more normal water balance (in that animals poisoned with barbital do not eat and very rarely drink water during the thirty to fifty hours after poisoning), and very probably reduces the tendency for the development of acidosis (Henze, 1936; Fischer and Salzer, 1936; Chang and Tainter, 1936; and Sack, 1936). Recovery is invariably characterized by the ingestion of water freely. Fluids alone are of definite symptomatic value in that the depth of lighter grades of hypnosis is diminished. The time required for complete recovery after such therapy alone appears to be little altered as observed by Riedel, 1930, although the mortality figures are significantly reduced. Optimal results are obtained when fluids are administered in conjunction with picrotoxin, metrazol, and ephedrine.

"Coramine," strychnine, and caffeine were of questionable value in the treatment of even lighter grades of barbital sodium poisoning. The order of observed efficiency corresponds to that in which the compounds are listed, as a matter of fact, the data obtained indicate undesirable effects, such as secondary depression from effective dosages, and, in the case of caffeine, these toxic effects were additive with those of the hypnotic.

CONCLUSIONS

The relative antidotal values of a series of analeptic measures have been tested on the rabbit after intraperitoneal medication with maximally sedative, maximally hypnotic, single lethal, and at least two lethal dosages of barbital sodium.

Control of the respiratory symptoms of poisoning by medication at suitable intervals for a period of thirty-six to forty-eight hours, with picrotoxin and metrazol, and of the circulation by means of ephedrine, together with fluids intravenously, prolonged the median period of survival after two lethal dosages of barbital from thirty-eight minutes (control) to a minimum of 39.5 hours and reduced the mortality from 100 per cent to 16.7 per cent.

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BACTERIOSTATIC ACTION OF PRONTOSIL SOLUBLE, SULFANILAMIDE, AND DISULFANILAMIDE ON THE SPORULATING ANAEROBES COMMONLY CAUSALLY ASSOCIATED WITH GASEOUS GANGRENE*

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IT IS impractical and unessential here to review the already voluminous literature on the nature and action of the sulfanilamide series in the treatment of various infections. This action has already been rather conclusively demonstrated experimentally against the streptococcus and pneumococcus (at least certain types), the gonococcus, the meningococcus, and more recently against malaria and the filtrable virus of lymphocytic choriomeningitis.

As far as we can ascertain, however, only one study records the clinical action against the sporulating anaerobes of human gaseous gangrene. Bohlman[1] reports the treatment with sulfanilamide of three cases with "an amazing, not to say, dramatic, result in a desperate case of gas gangrene. Cases 2 and 3 are confirmatory." *Clostridium welchii* was actually isolated only from Case 3, but was presumably present in all cases, either from symptoms or microscopic observation.

Although it is customary to precede clinical trial by some form of experimental in vitro test of a drug, no record of such test on the sporulating anaerobes

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of gaseous gangrene has yet been observed in the current literature. Bohlman does quote a personal communication from P. H. Long, who is stated to be "conducting experiments with *B. welchii* to determine the effect of Sulfanilamide on growth and phagocytosis."

In view of this lack of proof of direct, or primary bacteriostatic action of sulfanilamide on the sporulating anaerobes, it appears desirable to attempt such measurements of sulfanilamide and related compounds. To this end we obtained, through the courtesy of the Winthrop Chemical Company, Inc., experimental lots of prontosil soluble powder and of prontosil soluble (2.5 per cent solution). From Parke, Davis Company Research Laboratories we were provided with sulfanilamide and disulfanilamide.*

As test organisms we selected, from an extensive collection of sporulating anaerobes, a series of species commonly encountered, either alone or in combination, in human gaseous gangrene. These selected species were: *Cl. welchii, Cl. tertium, Cl. sporogenes, Cl. tetani, Cl. lentoputrescens, Cl. bifermentans, Cl. novyi, Cl. septicum,* and *Cl. histolyticum.* In addition was included *Cl. botulinum* Type A, although this organism is not associated with gaseous gangrene. Stock cultures of these organisms were grown in semisolid agar for twenty-four hours just preceding the tests.

Preliminary tests were conducted in a simple medium composed of 1 per cent Bacto-Tryptone, 1 per cent Bacto-Neopeptone and 0.2 per cent dextrose in distilled water. To this basic medium the several drugs were added in serial dilutions, which were then tubed in 7 by ½ inch tubes, each covered with a heavy oil seal 1 cm. deep, and autoclaved at 120° C. for twenty minutes. They were cooled quickly and inoculated immediately. The reaction of the medium, as inoculated, was about pH 6.8 to 6.9, and was not visibly altered by the drugs in the dilutions tested.

Each test dilution was inoculated, through the oil seal, with a Pasteur pipette from the twenty-four-hour-old stock culture in semisolid agar. Approximately 0.05 c.c. was delivered deep into each tube. All cultures were incubated at 37° C., and were observed at suitable intervals. Readings were recorded in terms of perceptible turbidity as compared with the control inoculation into the basic medium without added drug. The turbidity of the control culture is in all cases considered "4 plus," although the turbidity of *Cl. tetani,* for example, was much less than that of *Cl. sporogenes* or *Cl. bifermentans.* Inhibition of growth must, therefore, be judged by comparing each drug dilution culture with its own control.

The results of several repetitions are summarized in Table I. We should observe here that, while absolutely equivalent turbidities were not obtained, nor expected, on repetitions of tests of the same dilutions, there was a surprisingly high correlation.

From Table I it appears that prontosil soluble (2.5 per cent solution) exerts a direct bacteriostatic action, to and including a dilution of 1:1000, upon

*We desire to express appreciation to the Winthrop Chemical Company, Inc., and to Parke, Davis Company for the cooperation which made possible this study.

Cl. tetani, Cl. lentoputrescens, Cl. novyi, Cl. septicum and *Cl. histolyticum.* The other species tested were scarcely, if at all, affected until exposed to a concentration of 1:125.

It might be of interest to note that all species reduced the red color of the drug to a colorless state whenever growth occurred to a degree of marked turbidity. Some species were more active in this respect than others. Thus, in one test, *Cl. welchii* completely decolorized the 1:250 dilution, while *Cl. sporogenes* and others decolorized the 1:2000 but not the 1:1000 dilution.

Sulfanilamide exerted almost an identical degree of inhibition as did the prontosil soluble. Slight differences in turbidity are recorded, but these vary almost as much for one as for the other drug. Little, if any, more effective inhibition was observable with sulfanilamide, although such might have been expected from the chemical structure.

TABLE I

PRELIMINARY TEST OF SULFANILAMIDE, DISULFANILAMIDE, AND PRONTOSIL SOLUBLE IN DEXTROSE-PEPTONE WATER RECORDED AFTER 66 HOURS' INCUBATION

CULTURES TESTED	SULFANILAMIDE DILUTIONS							DISULFANILAMIDE DILUTIONS							PRONTOSIL SOLUBLE DILUTIONS							CONTROL NO DRUG
	1:125	1:250	1:500	1:1000	1:2000	1:4000	1:8000	1:125	1:250	1:500	1:1000	1:2000	1:4000	1:8000	1:125	1:250	1:500	1:1000	1:2000	1:4000	1:8000	
*Cl. welchii**	3	4	4	4	4	4	4	0	0	0	3	4	4	4	1	3	4	4	4	4	4	4
Cl. tertium	0	3	3	4	4	4	4	0	0	1	2	4	4	4	2	3	4	4	4	4	4	4
Cl. sporogenes	4	4	4	4	4	4	4	0	0	0	4	4	4	4	1	3	4	4	4	4	4	4
Cl. tetani	0	0	0	1	2	3	4	0	0	0	0	1	2	2	0	0	0	1	3	4	4	4
Cl. lentoputrescens	0	0	0	0	2	4	4	0	0	0	0	1	1	2	0	0	0	0	3	4	4	4
Cl. bifermentans	2	4	4	4	4	4	4	0	0	2	4	4	4	4	2	3	4	4	4	4	4	4
Cl. novyi	0	0	0	0	2	3	4	0	0	0	0	0	0	4	0	0	0	2	4	4	4	4
Cl. septicum	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4
Cl. histolyticum	0	0	0	0	1	3	4	0	0	0	0	1	4	4	0	0	0	1	4	4	4	4
Cl. botulinum Type A	2	3	4	4	4	4	4	0	0	1	4	4	4	4	1	2	3	3	4	4	4	4

*Numerals indicate degree of turbidity compared with that of the control.

Disulfanilamide, on the contrary, effected a distinctly superior inhibition, completely suppressing all species to and including a dilution of 1:250. Again, also, a specific action was apparent on the same organisms as were affected by prontosil soluble and sulfanilamide. Furthermore, this action was evident to higher dilutions, for example, *Cl. septicum* was completely inhibited by a 1:8000 dilution.

This test was then repeated, preparing dilutions from the prontosil powder. The results were essentially identical with those obtained with dilutions of the prontosil soluble (2.5 per cent solution), except that similar numerical dilutions of the powder appeared very slightly less effective than those derived from the prontosil soluble solution. This disparity, although slight, was uniformly and sufficiently evident to indicate a measurable and unexplained superiority of the action of the prontosil soluble (2.5 per cent solution).

In these above tests, it will be noted, the several drugs were added to the basic medium and sterilized by autoclaving. Since this is contrary to their

therapeutic use, it is conceivable that their activity may be depressed by such sterilization. At the same time, in this type of experimentation, some form of sterilization is, of course, essential. Moreover, the exigencies of anaerobic cultivation demand at least the boiling of the culture medium before inoculation, in order to insure conditions proper for growth. In this respect, then, the results of these experiments cannot be directly compared with those which may be obtained by administering the same drugs, unaffected by heating, either by mouth or by injection into the human body.

To determine if "self-sterilization" might be obtained, dilutions of 1:250, 1:500, and 1:1000 were prepared in the peptone broth, tubed under oil, and incubated without sterilization. After twenty-four hours' incubation, all tubes were heavily turbid with various contaminants. Since 1:250 marks approximately the saturation of disulfanilamide in the basic medium, this method was obviously eliminated.

TABLE II

DISULFANILAMIDE AUTOCLAVED IN DISTILLED WATER AND ASEPTICALLY ADDED TO THE BASIC MEDIUM RECORDED AFTER 66 HOURS' INCUBATION

CULTURES TESTED	DILUTIONS				
	1:1000	1:2000	1:4000	1:8000	CONTROL
Cl. welchii	3	4	4	4	4
Cl. tertium	0	2	4	4	4
Cl. sporogenes	3	4	4	4	4
Cl. tetani	0	2	3	4	4
Cl. lentoputrescens	0	0	3	3	4
Cl. bifermentans	2	4	4	4	4
Cl. novyi	0	0	0	0	4
Cl. septicum	0	0	0	0	4
Cl. histolyticum	0	0	0	0	4
Cl. botulinum Type A	3	4	4	4	4

Filtration of the initial dilutions might be attempted. However, unless boiled and protected against the reabsorption of oxygen, the addition of these dilutions would reaerate the test mediums. Due to these, and other technical complications, filtration experiments were not attempted.

We did, however, utilize a principle commonly applied to the sterilization of certain carbohydrates. The disulfanilamide was autoclaved in a 1:125 dilution in neutral distilled water. The basic peptone broth was tubed under oil in 9 c.c. amounts and autoclaved separately. This dilution of disulfanilamide is really a supersaturated solution, but remains in solution while hot. While still in solution, serial dilutions were made with sterile pipettes through 9 c.c. sterile distilled, and freshly boiled, distilled water blanks. These dilutions were then aseptically delivered, in 1 c.c. amounts, under the oil seal into the basic medium to effect the desired end dilutions. These tubes were then inoculated and incubated. The results are shown in Table II.

In comparing the results expressed in Table II with those in Table I it appears, superficially, that the autoclaving of disulfanilamide in distilled water

permits it to retain a slightly greater bacteriostatic activity. However, again the factor of necessary introduction of oxygen with the aerated dilutions of the drug might be sufficient to account for this.

It is a simple matter, using methylene blue as an indicator, to show that boiled distilled water becomes fully aerated within the time required to make the necessary dilutions and to deliver these into the basic medium. It would be difficult to evaluate this possible cause, but at least no appreciable gain is observed by autoclaving the drug in distilled water, with subsequent addition to the medium.

Obviously these in vitro tests in a simple peptone medium do not approach the complexity of conditions within the human body. Nor is there any practicable way to attain them under conditions suitable for anaerobic cultivation.

TABLE III

TEST OF SULFANILAMIDE, DISULFANILAMIDE, AND PRONTOSIL SOLUBLE (2.5 PER CENT SOLUTION) IN DILUTE LOEFFLER'S SERUM-DEXTROSE-PEPTONE BROTH RECORDED AFTER 66 HOURS' INCUBATION

CULTURES TESTED	SULFANILAMIDE DILUTIONS						DISULFANILAMIDE DILUTIONS								PRONTOSIL SOLUBLE DILUTIONS						CONTROL NO DRUG
	1:250	1:500	1:1000	1:2000	1:4000	1:8000	1:250	1:500	1:1000	1:2000	1:4000	1:8000	1:16,000	1:32,000	1:250	1:500	1:1000	1:2000	1:4000	1:8000	
Cl. welchii	1	2	4	4	4	4	0	0	1	2	4	4	4	4	1	2	4	4	4	4	4
Cl. tertium	0	0	1	2	4	4	0	0	0	0	1	2	4	4	0	1	2	4	4	4	4
Cl. sporogenes	3	4	4	4	4	4	0	3	4	4	4	4	4	4	3	4	4	4	4	4	4
Cl. tetani	1	2	3	3	4	4	0	0	0	0	1	2	3	4	0	1	2	4	4	4	4
Cl. lentoputrescens	0	1	2	2	4	4	0	0	0	0	0	1	2	2	0	1	2	3	3	4	4
Cl. bifermentans	1	2	4	4	4	4	0	0	0	1	2	4	4	4	1	2	4	4	4	4	4
Cl. novyi	0	0	0	1	3	3	0	0	0	0	0	1	2	4	0	1	2	3	3	4	4
Cl. septicum	0	0	1	2	4	4	0	0	0	0	1	2	4	4	0	0	1	2	4	4	4
Cl. histolyticum	1	2	3	4	4	4	0	0	1	2	3	4	4	4	0	2	4	4	4	4	4
Cl. botulinum Type A	3	4	4	4	4	4	0	1	3	4	4	4	4	4	2	4	4	4	4	4	4

We did simulate them, to a degree, by preparing a second basic medium composed of Difco Loeffler's blood serum dehydrated. One gram of this powder was dissolved in 140 c.c. of the stock dextrose-peptone broth. In this medium the several drugs were diluted as before, tubed under oil, and autoclaved. The tubes contained, after autoclaving, a fibrinlike clot in a clear fluid.

Since disulfanilamide, in the previous tests, completely inhibited certain species to the maximum 1:8000 dilution, in this test it was diluted further to 1:32,000.

The tubes were inoculated, incubated, and observed for turbidity and decolorization of the prontosil soluble. The results are recorded in Table III.

When Table III is compared with Table I, it is evident that the action of the several drugs in dilute Loeffler's serum-water is practically identical with that in the simple dextrose-peptone broth. If anything, the activity in the serum-water is slightly less, as noted particularly with the disulfanilamide. At least there is no distinct increase, such as is stated to occur in tests of acriflavine and certain similar compounds.

Finally, a series of tests was run in a semisolid agar prepared by adding 0.25 per cent agar to the dextrose-peptone water base. The several drugs were diluted as before and tubed without oil seal, autoclaved, inoculated, and incubated.

The readings in this semisolid agar so nearly exactly parallel those in the previous tests that it is not essential to present them in tabular form.

SUMMARY

We have attempted to measure, under several experimental conditions, the direct bacteriostatic action of sulfanilamide, disulfanilamide and prontosil soluble upon a series of sporulating anaerobes commonly causally associated, alone or in combination, with human gaseous gangrene.

These tests indicate that, under these test conditions, the drugs exert an apparently specific selective action against certain species, notably *Cl. tetani*, *Cl. lentoputrescens*, *Cl. novyi*, *Cl. septicum*, and *Cl. histolyticum*. On the contrary, *Cl. welchii* and the intensely proteolytic types such as *Cl. sporogenes* and *Cl. bifermentans*, as well as the toxic *Cl. botulinum* Type A, are scarcely at all affected, at least by any concentrations approaching those tolerated in therapeutic dosages of human beings.

It appears, from these tests, that the bacteriostatic activity increases in order: prontosil soluble, sulfanilamide, and disulfanilamide. This is in keeping with their chemical composition, and might be anticipated. In fact these results parallel those of Rosenthal, Bauer and Branham[2] in testing these same compounds for protection of mice against streptococcus and meningococcus infection.

Lastly, admitting Bohlman's[1] "dramatic" results in the treatment of 3 cases of presumably *Cl. welchii* infections with sulfanilamide, and comparing the experimental concentrations tested with the maximum estimated body concentrations attained by his dosages, it would seem necessary to assume some secondary therapeutic activity of the drug other than a simple and direct bacteriostatic action upon the bacteria themselves.

These experiments measure only the direct bacteriostatic activity, and we attempt here no explanation of the obvious discrepancy between the experimental results and the therapeutic records.

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THE URINARY EXCRETION OF SINGLE DOSES OF SULFANILAMIDE*

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THE successful treatment of urinary infections[1-5] with sulfanilamide has directed attention to the measurement of urinary concentrations of the drug[2, 3] since it has been pointed out that the therapeutic efficacy is a function of the concentration of the drug in urine. With a view to further elucidation of the elimination of the drug, we have administered single doses to three male subjects. F., a normal white male, weighing 125 pounds, was given 2.0 gm.; L. T., a normal colored male, weighing 150 pounds, was given 2.5 gm.; and A. F., a normal white male, weighing 125 pounds, was given 3.0 gm. A. F. became dizzy and nauseous three hours after ingestion of the drug. He forced fluids and was given 1.0 gram of sodium bicarbonate. These subjects were occupied with their normal routines. Urine samples were voided ad libitum, and analyzed for free and total sulfanilamide.

The data obtained are abbreviated in Table I to show only the maxima observed, and the number of hours after ingestion of the drug at which these occurred. Concentrations are expressed in milligrams per cent, and rates in milligrams per hour.

The rate of excretion reached apices which did not correspond to the concentration peaks, although maximal rates of excretion of free and total sulfanilamide occurred at the same time in all cases. In two cases an additional later peak was observed in the rate of total sulfanilamide excretion.

The maxima observed for conjugated sulfanilamide concentrations occurred later than the peaks for the free sulfanilamide. Consequently, the concentration maxima of the total sulfanilamide correspond to the maxima of the free, or conjugated sulfanilamide, or both.

The multiple peaks observed rise sharply, and are undoubtedly the result of the factors, viz., water intake, urinary pH, physical activity, tissue desorption, tubular resorption,[6] etc.

Excretion of the drug, in the order of the cases presented, was complete in fifty, fifty-eight, and fifty-four hours. The total recovery of sulfanilamide was 53, 51, and 58 per cent of the amount ingested, respectively. Over all values were checked by diazotizing and coupling the combined urine samples in each case to overcome the colorimetric reading errors at low concentrations. The data were obtained in the third case, using Marshall's method,[7] and in all other cases using the chromotropic acid method.[8] The total recovery is in good agreement with the data obtained by Fuller[9] in animals. Approximately 45 per cent of the initial dose of sulfanilamide remains unaccounted for. This

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may be partially excreted in another form, since Fuller could find only small amounts in feces, and Marshall[10] recovered less than 2 per cent in the intestinal tract of dogs, four hours after ingestion of the drug.

The recovery of free sulfanilamide was 24, 17, and 37 per cent. The last value appears to be high and may be due to the diuresis induced.

TABLE I

SULFANILAMIDE

CASE	FREE				CONJUGATE		TOTAL			
	CONCENTRATION MAXIMA MG. %	TIME ATTAINED (HOURS AFTER INGESTION)	RATE MAXIMA MG. PER HOUR	TIME ATTAINED	CONCENTRATION MAXIMA MG. %	TIME ATTAINED (HOURS AFTER INGESTION)	CONCENTRATION MAXIMA MG. %	TIME ATTAINED (HOURS AFTER INGESTION)	RATE MAXIMA MG. PER HOUR	TIME ATTAINED
F.	35.1	3	40.3	3	26.3	7	61.2	17	58.6	4
	36.1	19	48.8	19	35.0	19	43.6	26	96.0	17
					28.4	26			24.0	26
L. T.	32.4	11	26.3	6	34.4	11	66.8	17	42.0	6
			21.4	33	45.1	48	56.8	48	63.3	33
A. F.	68.9	4	123.0	8	21.4	6	81.6	6	131.0	8
	55.1	19			31.1	19	86.2	19	50.0	19
					27.7	28	46.8	28		

It was noted that approximately 80 per cent of the free sulfanilamide excreted (80.0, 87.5, 75.4 per cent) was recovered during the first twenty-four hours, whereas only 60 per cent of the total sulfanilamide (65.0, 57.5, 63.1 per cent) was excreted during this time. The difference is due to a lag in the excretion of acetylsulfanilamide and was anticipated from the recession of the concentration peaks observed. This may result from a time requirement in the detoxification mechanism.

TABLE II

CASE	URINE VOLUME IN 1ST 24 HOURS	FREE SULFANILAMIDE IN GRAMS IN 1ST 24 HOURS	% RECOVERED AS FREE SULFANILAMIDE IN 1ST 24 HOURS	TOTAL GRAMS IN 24 HOURS	% RECOVERED OF TOTAL IN 1ST 24 HOURS
A. K.	857	0.181	3.6	0.412	8.2
J.	995	0.445	11.4	0.791	19.8
T.	2020	0.370	14.8	0.725	29.0
H.	2250	0.980	19.6	1.495	29.9
F.	1745	0.385	19.2	0.688	34.4
A. F.	2430	0.836	27.9	1.100	36.6

Since colorimetric comparison is more accurate at higher urinary concentrations of sulfanilamide, the following three male patients, recovering from tibia-fibula fractures, were studied during the first twenty-four hours only. The amount of sulfanilamide administered in these cases was increased to 4 and 5 gm. Helmholz and Osterberg[2] report that after a single 5-gram dose of the drug, the urine will at some time contain as high as 60 mg. per cent free and 100 mg. per cent conjugated sulfanilamide. One patient, A. K., weighing 130 pounds, and given 5 gm. of the drug, did not nearly approximate

these values. H., weighing 180 pounds, given 5 gm., achieved a free sulfanilamide concentration of 75 mg. per cent, but the conjugated form did not exceed 50 mg. per cent. J., weighing 168 pounds, and given only 4 gm., fell in this range. A fourth case, G., not reported here in detail, with obvious kidney involvement, and given only 5 gm. achieved concentrations of 80 mg. per cent free and 175 mg. per cent conjugated sulfanilamide.

Care was exercised to avoid urine losses in all cases. The recovery within the first twenty-four hours of the free and total sulfanilamide expressed in per cent of the drug administered (see Table II) varied widely. It was observed that the low recovery was concomitant with low urinary volume.

The retention of sulfanilamide in the first two cases (A. K. and J.) is apparently not related to impaired kidney function, since both subjects were regarded clinically normal. The high value of free sulfanilamide in Case A. F. (27.9 per cent) may be due to diuresis. These findings are in agreement with the results reported by Marshall.[6] The amount of the drug excreted during the first twenty-four hours is obviously not solely related to the urinary volume.

SUMMARY

Multiple peaks have been observed in the concentration and the rate of output of free and conjugated sulfanilamide in the urine following single doses of the drug. The rate and concentration maxima do not appear to be related.

Fifty-five per cent (±5 per cent) of the ingested sulfanilamide has been recovered.

The percentage of the drug recovered within the first twenty-four hours varied from 8 to 36 per cent. The two lowest values were, among other things, associated with a low urinary output and were apparently not related to impaired kidney function.

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LABORATORY METHODS

A MICROCONSTANT EXTRACTION APPARATUS FOR LIQUIDS*

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IT IS frequently necessary to extract a small amount of fluid with a nonmiscible solvent under conditions where mechanical separation is unsatisfactory. A wide variety of devices has been described for the automatic constant extraction of liquids with lighter, nonmiscible liquids;[1, 2] but none is suitable for the extraction of volumes under 10 c.c., and most achieve efficiency at the cost of simplicity. From the latter standpoint, the apparatus described by Kurzrok and Ratner[3] is an exception, and has formed the basis of design for the microapparatus described here.

The use of such an apparatus for the extraction of small amounts of fluid quantitatively has a threefold advantage over the use of a separatory funnel: The extraction is not attended with the mechanical losses encountered with the funnel in all but the most skilled hands; the extract is left in a small volume of solvent; and, the process being automatic, it is less time-consuming. Although the following description is somewhat specific in respect to details, the apparatus (since it can be made by anyone with reasonable skill in glass-blowing) lends itself to wide variation, adapted to specific needs.

Referring to Fig. 1, it will be seen that the apparatus consists essentially of a boiling chamber, *A*; a condensing chamber, *B*, fitted with a condenser; and an extracting chamber, *C*. The boiling chamber is fitted with an outlet tube, *E*, leading to *B*, and a return tube, *F*, leading from *C*. The condensation chamber terminates below in a narrow tube, *H*, reaching to the bottom of *C*, and carrying a sealed concentric outlet tube, *G*, into which *F* is fused.

The boiling chamber, in the case illustrated, is a 125 ml. Erlenmeyer flask; and the extraction chamber, a test tube 6 inches by ¾ inch. The condensation chamber is of 25 mm., and the vertical tube *H*, of 5 mm. glass tubing. The joint between *B* and *H* is best made with an intermediate section of tubing of larger diameter than *H*, as illustrated, for the sake of sturdiness. A bulb is blown on the lower end of *H*, containing minute perforations in its undersurface. All stoppers are cork of good quality. Construction is easier, and the apparatus less fragile, if tube *E* is made discontinuous, and provided with a joint of rubber tubing (*D*).

In operation, 50 to 75 c.c. of solvent is placed in *A*, and the fluid to be extracted, in *C*. The latter is then filled nearly to the top with solvent. The apparatus is connected, the condenser water started, and the solvent in flask *A*

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boiled over an appropriate source of heat. Vapor from the solvent reaches *B* through *E;* is condensed, and collects in *H* until the height of its column overbalances the pressure exerted by fluid and supernatant solvent in *C*. Then the solvent flows out through the perforations in the bulb at the end of *H*, passes up through the fluid in a fine state of subdivision, extracting as it goes; and joins the supernatant solvent. When the latter has reached the level of the return tube *F*, it flows back into *A*, carrying with it extracted material from *C*.

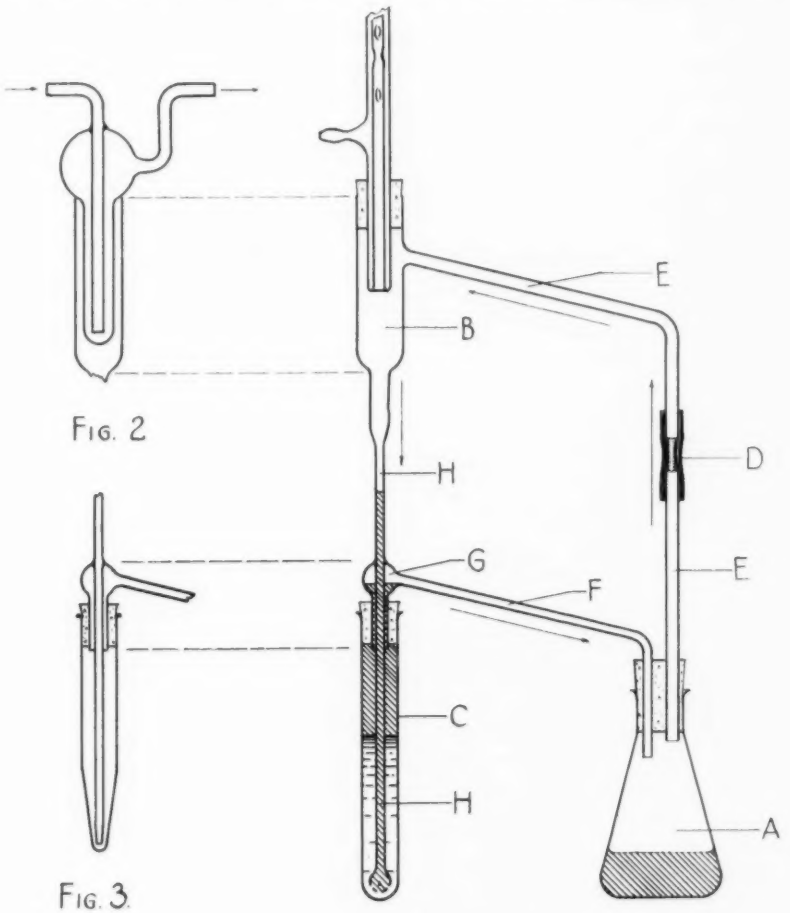

FIG. 2

FIG. 3

FIG. 1

Figs. 1, 2, 3.

The fluid is being continually permeated by a stream of fresh solvent, and the extracted matter is eventually all concentrated in *A*.

At the end of the extraction (which usually occupies fifteen to thirty minutes) the extract may be recovered by evaporating the solvent in *A*, and may then be measured by appropriate means. To recover the extracted fluid, tube *H* is withdrawn from the extraction chamber, washed inside and out, and as much of the supernatant fluid as possible drawn off with a pipette. The remaining solvent may be driven off by warming *C* in a water-bath.

Some variants of the above are illustrated in Figs. 2 and 3. In Fig. 1, a 395 mm. West condenser is shown, which experience has proved to be most satisfactory when using ether as a solvent. When solvents of higher boiling point, such as ethyl acetate, are employed, the condenser illustrated in Fig. 2 is adequate, and has the advantage both of lowering the center of gravity of the assembly, and of eliminating one cork joint. This condenser, which may be blown from a test tube, slips loosely into the mouth of the condensation chamber. Under some circumstances, a 15 ml. centrifuge tube may advantageously be used as an extraction chamber (Fig. 3). In this case, the bulb at the end of *H* is omitted, and the tube drawn to a blunt taper. Instead of the concentric sealed joint, *G*, tubes *H* and *F* may pass through separate holes in a cork at the mouth of *D;* however, it is difficult to make a sufficiently tight joint in this way. *A*, *B*, and *C* could easily be equipped with standard taper ground joints in an apparatus intended to be used extensively.

Two illustrative examples of the application of this apparatus may be cited from the author's experience. In attempting to extract quantitatively the lipids from a chylous fluid,[4] using an alcohol-ether mixture, a stubborn emulsion was encountered upon shaking. Using the apparatus illustrated in Fig. 1, the extraction was accomplished in fifteen minutes without difficulty. In decomposing the picrates of the guanidine bases, the modification shown in Fig. 3 has been especially useful, and the method lends itself to the decomposition of other similar basic derivatives. The picrate was precipitated in 15 ml. centrifuge tubes, and centrifuged. The mother liquor was decanted and the tubes allowed to drain. Then 5 to 7 c.c. of 10 per cent hydrochloric acid was added to the crystals, and the suspension extracted with ethyl acetate for thirty minutes. The base remained as the hydrochloride in the centrifuge tubes, and could be determined manometrically by the hypobromite method. Guanidine, in amounts of 1 to 5 mg., was recovered to the extent of 95 to 98 per cent; and *as*-dimethyl guanidine, in like amounts, gave a uniform recovery of 75 per cent by this method.[5]

SUMMARY

An easily-constructed apparatus is described, which permits the constant extraction of 1 to 10 c.c. of fluid with a lighter, nonmiscible solvent. The apparatus may readily be modified to suit specific requirements.

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COCK CONTROLLED AT A DISTANCE FOR EXPERIMENTAL STUDY OF BREATHING*

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ALL experimental researches on the respiratory gases, basal metabolism determinations, alveolar gas samples, determinations of cardiac output, lung capacity, etc., require the use of a cock with three or more "ways" which gives the patient's mouth communication either with outside air, or with spirometers, gasometers, bags, etc.

The cock works by means of a handle attached to it and is turned by the hand of the operator. This is done very close to the patient's face, causing him disturbance which is shown by his altered breathing activity. Moreover, a very

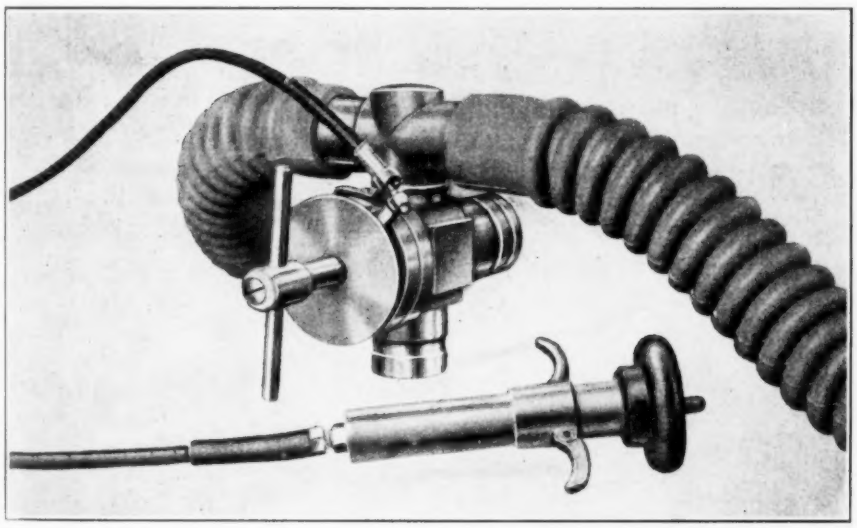

Fig. 1.

skillful operator is required in order to watch both the breathing activity of the patient and the stopwatch, besides other experimental particulars.

In order to reduce the work of the operator, I have designed a cock which may be manipulated with remote control (Fig. 1).

It has three outlets. In a fixed position a winding spring connects two outlets. By a turn of the handle the bore of the cock will move through an angle of 90°, one of the outlets will be plugged and the other two will be connected; at the same time the winding spring will be stretched; When the handle is released, the winding spring brings the cock to its first position.

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This article may be obtained from Ing. G. Terzano & C., Milan, Italy.

The first turn of the handle can be executed at a distance by means of a flexible transmitter of Bowden type, which is controlled by a hand stretcher with a stop hook. This keeps the cock permanently in the turned position.

In order to go back to the first position, switch off the stop hook, and the winding spring will automatically turn the handle.

With this device, the operator can place himself beside or behind the patient and work the cock with one hand in a very simple and sure way, leaving it opened afterwards either in one or in the other position for the time required.

CRYSTAL TESTS FOR MINUTE AMOUNTS OF MORPHINE*

CHARLES C. FULTON, ST. PAUL, MINN.

THE reagents to be described provide crystal tests applicable to less than one gamma, one-millionth of a gram of morphine. All are iodine reagents, but they differ in composition and properties and in the crystals they yield with traces of morphine. There are four distinct types of crystals: (1) black needles, (2) rosettes of brown threads, (3) tiny rectangular orange brown rod plates, and (4) much larger red square-cut plates. Six reagents are given; I and II are made with different acids, but yield the same crystals, the black needles. The next two reagents yield the brown threads; with III these vary slightly towards the black needles, and with IV they are associated with the tiny orange brown rectangular crystals. Reagent V yields the tiny rectangular crystals, still associated with some scattered straggling threads; VI yields the familiar red plate crystals, which are commonly considered the most characteristic morphine crystals.

With larger amounts of morphine, in fact, all these reagents yield the plate crystals, where the concentration is suitable; the first three giving chiefly rosettes of brown threads in the outer parts of the test drops where the concentration of morphine is less.

The reagents are not for morphine alone. They give crystals readily with many different amines and alkaloids, and can be used for numerous identifications. Their other uses, however, have not yet been studied; nor do we even know which of the crystals are most characteristic of morphine in testing traces, nor which are formed most readily if the morphine is not perfectly pure.

TECHNIQUE

Add the reagents directly to a little of the dry alkaloid or its salt.

Evaporate a drop of solvent containing morphine on the microscope slide. (Chloroform solutions were used in the investigation—the morphine was dissolved in a little alcohol and dilute solutions made up with chloroform.) Do not allow the drop to spread all over the slide; evaporate it in a small area,

*From the Alcohol Tax Unit, St. Paul Laboratory.
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say by blowing on it. If necessary, evaporate several or any number of drops in the same place. If the amount of morphine suspected is no more than one gamma, draw a line on the slide with the grease pencil pointing to the deposit and ending near it. Add a drop of reagent; then a cover glass (¾ inch), squarely over the deposit. This spreads the reagent evenly and thinly over the deposit and the adjacent slide. Crystallization usually begins in about fifteen minutes, is usually well developed within thirty to forty-five minutes, and about as complete as it will ever be in an hour or an hour and a half. The low power of a good microscope is generally adequate, except for the tiny rectangular crystals given by reagents IV and V. These merit examination under high power.

FACTORS CAUSING DIFFERENT EFFECTS OF THE REAGENTS

The reagents are made by diluting specified I-KI solutions with diluted or mixed acids. The I-KI solutions can be kept in stock; the reagents themselves are not entirely permanent, but a preparation can be used for two or three days, even if left in an open test tube.

The effect of a reagent depends on the ratio of KI to iodine, or the excess of KI over that needed to hold the iodine in solution, and on the acid used. Excess of KI has a solvent effect on the morphine-iodine compound, besides changing the crystals from one type to another in the order already mentioned. Hydrochloric and hydrobromic acids slightly increase the solubilities of iodine and of the morphine-iodine compound, and acetic acid increases them greatly; sulfuric acid slightly decreases them, and phosphoric acid decreases them to a great extent. Thus with phosphoric acid more KI is needed just to hold the iodine in solution than with the other acids, while in spite of the increased KI, the morphine-iodine compound is less soluble. Aside from solubilities, hydrochloric acid has a tendency to favor crystallization in black needles; phosphoric acid favors brown threads; and sulfuric acid is more favorable than the others to the formation of plates, but is also good for black needles.

One hundred and forty reagent formulas were tested in the experiments. It may be found that the crystals vary with still other factors, particularly temperature. The experiments were carried out at a room temperature of about 22° C.

The following reagents are recommended:

Iodine Reagent M-I.— I-KI solution: Iodine 10 gm.
KI 50 gm.
Water to make 100 c.c.
Reagent—Mix 1 c.c. of the I-KI solution with 4 c.c. of (1 plus 3) H_2SO_4.
Crystals—Black needles, more or less branching and in thin rosettes. Good crystals are obtained with 0.1 gamma.

Iodine Reagent M-II.— I-KI solution: Iodine 5 gm.
KI 30 gm.
Water to make 100 c.c.
Reagent—Mix 2 c.c. of the I-KI solution with 3 c.c. concentrated HCl and 3 c.c. syrupy H_3PO_4.
Crystals—Black needles, mostly in thin rosettes. Good crystals are obtained with 0.1 gamma.

This reagent possibly gives the most rapid crystallization, closely followed by reagents I and III.

Iodine Reagent M-III.— I-KI solution: Iodine 7.5 gm.
KI 75.0 gm.
Water to make 100 c.c.

Reagent—Mix 1 c.c. of the I-KI solution with 1.5 c.c. water and 2.5 c.c. syrupy H_3PO_4.

Crystals—Dark brown threads in rosettes, varying to black needles. Good crystals are obtained with 0.1 gamma.

Iodine Reagent M-IV.— I-KI solution: Iodine 5 gm.
KI 80 gm.
Water to make 100 c.c.

Reagent—Mix 1.5 c.c. of the I-KI solution with 2.5 c.c. water and 3.5 c.c. syrupy H_3PO_4.

Crystals—Light brown threads in rosettes, interspersed with a few small orange brown rectangular rod plates. The latter become larger and more numerous on standing. Crystals with 0.25 gamma.

Iodine Reagent M-V.— I-KI solution: Iodine 7.3 gm.
KI 60.0 gm.
Water to make 100 c.c.

Reagent—Mix 1 c.c. of the I-KI solution with 4 c.c. of (1 plus 3) H_2SO_4.

Crystals—A few straggling brown threads, and many tiny rectangular rod plates, mostly brownish orange to orange brown under high power. Generally some of the threads form first; on standing most of the crystallization is in the form of the tiny rectangular crystals. Crystals form with 0.25 gamma.

As an alternative, this reagent can be made with the I-KI solution given for III, mixing 1 c.c. with 4 c.c. of (5 plus 11) H_2SO_4. Decant from the $KHSO_4$ that gradually crystallizes out.

Iodine Reagent M-VI.— I-KI solution: Iodine 5 gm.
KI 80 gm.
Water to make 100 c.c.

Reagent—Mix 1 c.c. of the I-KI solution with 4 c.c. of (5 plus 11) H_2SO_4. Decant from the $KHSO_4$ that gradually crystallizes out.

Crystals—The square-cut deep red plates which we generally consider the characteristic morphine-iodine crystals. The color may vary to orange brown. Crystals form with 0.5 gamma; they are of good size with as little as 1 gamma. These crystals require a longer time to form in any number than those with the preceding reagents.

IDENTIFICATION OF TRACES OF MORPHINE

Heretofore the most sensitive identity test known for morphine has been the one with Buckingham's reagent (10 to 15 gm. ammonium or sodium molybdate in 100 c.c. concentrated H_2SO_4, a permanent reagent as long as it is protected from the moisture of the air). This reagent, a variety of Fröhde's, gives an intense purplish red color which, after a short time, fades out. In a previous

comparative study of the tests for morphine, the writer found that Buckingham's reagent gives an unmistakable reaction with 0.4 gamma. As a check on the sensitivity of the new crystal tests, the sensitivity with a single drop of Buckingham's reagent was redetermined, using the same solutions used to give deposits of morphine for the crystal tests. In these tests, 0.3 gamma gave a purplish pink; 0.2 gamma a "doubtful" pinkish color; 0.1 gamma a negative reaction, or a faint and very doubtful evanescent pinkish color. The crystal tests with reagents I or II, and III, are, therefore, the most sensitive identity tests now known for morphine, and the crystal tests with reagents IV and V are at least as sensitive as Buckingham's color test. Crystallization is perhaps more likely to fail from unknown or uncontrollable factors, but affords a more definite, less subjective test than a color.

It is now possible by two entirely independent tests to identify as little as one gamma of morphine, or even less, provided it can be isolated in two small deposits, one on the spot plate and one on the microscope slide. One of these tests, with Buckingham's reagent, is a characteristic color test for a particular phenol—morphine; the other, with one of the iodine reagents given herein, is a characteristic crystal test for a particular amine—again morphine. The identification of enormously larger quantities of most substances is in general based on tests less independent of each other, less characteristic, and less certain than these that identify morphine in the amount of a millionth of a gram.

FRÖHDE'S REAGENT: A REAGENT FOR MORPHINE AND FOR OTHER PHENOLIC COMPOUNDS*

CHARLES C. FULTON, ST. PAUL, MINN.

FRÖHDE introduced his reagent as a test for morphine in 1866, but in spite of its great importance and the long lapse of time, completely accurate accounts of the reagent itself, its morphine reaction, and its effect on other compounds have been entirely lacking.

The reagent is molybdate in concentrated sulfuric acid. It is made by dissolving about 0.5 gm. sodium or ammonium molybdate in 100 c.c. concentrated sulfuric acid. More than 1 gm per 100 c.c. should not be used, except for the definite variation, "Buckingham's reagent." As little as 0.1 gm. (1 mg. per cubic centimeter) is sometimes used. Solution is readily effected by warming the acid on the water bath, in fact it can even be obtained at room temperature. Molybdic anhydride will not dissolve so readily. Its solution, however, can be effected by digesting at about 160° C., and it then gives the same reactions.

*From the Alcohol Tax Unit, St. Paul Laboratory.
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MORPHINE

Added to a little morphine (the free alkaloid or the sulfate) on the spot plate, Fröhde's reagent gives an intense bright purplish red color, after a little time (about four minutes) fading and passing through a comparatively weak brown or brownish, or with a very small quantity of morphine even fading out completely, then developing a strong bright green. On the spot plate this color ordinarily changes on standing to an olivaceous yellow brown or brown yellow. This is due to absorption of moisture from the air. With several cubic centimeters in a test tube, the green solution, in the course of about two hours, becomes a pure deep blue. In a hot dry atmosphere, or if set in a desiccator, the spot-plate test may show, for a time, a bluish green to bluish slate color.

The reaction may be developed by heat. Ordinarily this is inadvisable, for impurities are more likely to react, or if they do not react with the molybdate, may be charred or otherwise decomposed by the hot sulfuric acid, spoiling or obscuring the true colors of the reaction. However, heating may sometimes be of value in testing traces. After adding a drop or two of the reagent, the spot plate is immediately set on the boiling water-bath. The purplish red color quickly fades and passes into the green, and with further heating is changed to slate blue. More heating may change the color to brown; this takes place more quickly the greater the amount of morphine.

Fröhde's reagent is very sensitive. A single drop, applied to a small dry deposit of morphine, strikes a noticeable purplish pink, quickly fading, with 0.001 mg. of morphine. In a dry atmosphere, the green color will develop with 0.003 mg. In the heating test the three colors, purplish pink, green, and slate blue are obtained with 0.002 mg. In a very humid atmosphere, the reagent may absorb moisture too rapidly to show the green color with only a minute trace of morphine. However, under the most adverse conditions, stirring the alkaloid into solution in a "spot" full of reagent, the green color develops throughout the solution within a few minutes of the time the red fades out, with no more than 0.02 mg.

With sulfuric acid that is not quite full strength, the initial color is slightly bluer, varying from purple red to reddish purple.

When made with fuming acid (15 per cent free SO_3), the reagent strikes dark red, which fades almost instantly; the solution becomes colorless or slightly brownish; a green soon develops, and changes to dirty brown. "Fuming Fröhde's" is an extremely valuable reagent for the opium alkaloids in general, although the morphine reaction is not improved by its use.

With the hydrochloride of morphine, Fröhde's reagent strikes a violet color (accompanying the effervescence of HCl); but this quickly changes to the same purplish red given by the free alkaloid or the sulfate, acetate, etc. The color then passes through a weak brown to an olivaceous green, which persists, even in the test tube, as an olive green, long after the sulfate solution (in the test tube) has turned blue.

Heroin reacts like morphine; codeine, dionin, and papaverine give feeble reactions, green changing to blue, with the ordinary Fröhde's reagent. The

statement met with in some texts that papaverine reacts like morphine is entirely contrary to the observations of the writer. The "Fuming Fröhde's" reagent does, however, give a test of great value, an intense violet color, with papaverine.

DESCRIPTIONS IN THE TEXTS

Fröhde's morphine test is one of the best and most characteristic known, but the errors, myths, and misdescriptions that fill the texts concerning it are as remarkable as it is.

First, one is usually directed to make the reagent up fresh, and the statement is frequently made that it will not keep. Fröhde's reagent is just as permanent as the sulfuric acid from which it is made. In a glass-stoppered bottle, or even a rubber-bulb dropping flask, it will keep indefinitely; but exposed to the air, the acid absorbs water and becomes diluted and worthless.

Second, the myth persists, and is found in most texts, that the colors are due merely to "lower oxides" of molybdenum. That molybdenum enters into the colored compounds is probable, but that the colors are due solely to the reagent and not to the substance tested is quite impossible. The Peterson, Haines, and Webster Toxicology, as an exception, does have the following as a footnote: "The play of colors obtained by the action of morphine upon the molybdic acid, under the anhydration of the sulfuric acid, is so characteristic of a very few organic compounds, that it must be due to the formation of organic derivatives."[1]

The conclusion is certain from the almost unlimited variety of colors produced and the nature of the reactive compounds, which are generally of phenolic character (true phenols or ethers of phenols), or aromatic amines.

To be sure, if a too concentrated molybdate solution is used, a deep sapphire blue color develops at the edge and gradually pervades the whole solution; and since this occurs not only with morphine but with all the reactive compounds, this particular color is no doubt due to the molybdenum. But it is not likely that this reservation even includes the blue that develops in the test tube, in the reaction as described above.

Third, is the confusion in regard to Buckingham's reagent. Most texts distinguish it from Fröhde's as being made with ammonium molybdate instead of sodium molybdate or molybdic acid. This is no distinction at all. Mulliken even makes Buckingham's reagent with less molybdate than Fröhde's; but Prescott[2] and Fresenius[3] correctly explain the distinction, which is that Buckingham used more molybdate. Buckingham's reagent can be made with sodium molybdate, but ammonium molybdate contains less water of crystallization in proportion, and, therefore, may be a little better.

Fourth, is the description of the reaction. With Fröhde's reagent on the spot plate (at room temperature), the color is never a true blue, and rarely shows any blue or bluish color at all. Nevertheless, the descriptions in the texts, various as they are, chiefly stress a blue color, and even place it before the green, when they mention a green color at all. They also insist upon a "violet" color. This much abused color term is used sometimes for a purple red, magenta,

or similar color; but since some descriptions call for "purple changing (or fading) to violet" or "blue violet"—"passing into blue then green"[4, 5] it can hardly be supposed that by "violet" is meant a red hue, especially as still other descriptions have (instead of "violet") "purple changing to blue."[6, 7]

It is impossible to dismiss these descriptions entirely, and it is equally impossible to accept any of them as correct. It cannot even be said that the older descriptions are the less accurate, for even in 1875, Taylor described the initial colors correctly as "intense reddish purple or deep crimson, this changing to dingy green,"[8] and twenty years ago, Mulliken gave a substantially correct description of the spot-plate test.[9]

On the whole it appears that the blue color which causes the confusion, sometimes dominating the reaction and sometimes not appearing at all, must be caused by some unknown impurity, perhaps one formerly present—sometimes—in the sulfuric acid. Presumably, too, this interfering blue color is due primarily to the molybdenum rather than the morphine, although the morphine as a reducing agent may help produce it.

PHENOLIC COMPOUNDS

Reactive compounds are, in general, phenols or ethers of phenols, or, in some cases, aromatic amines. All kinds of colors and sequences of colors are produced. No known compound reacts exactly like morphine, and the reactions of many other substances are equally specific.

Some phenols give a purplish red, lavender, or purple color, more or less like that produced with morphine. These include arbutin, hydroquinone, vanillyl alcohol, pyrocatechin, orcinol, carvacrol, and thymol. The reactions of a few such compounds (carvacrol, hydroquinone) might be described in general terms rather like that of morphine; nevertheless, it is safe to say that with a side-by-side comparison, or subject to long familiarity with the morphine reaction, it would be nearly impossible to mistake one of the other reactions for that of morphine.

Poe and Stehley recently reported on the examination of a large number of compounds with Fröhde's reagent and came to the conclusion: "In general, the compounds giving a similar color, as does morphine, with this reagent, do not belong to any definite group of organic compounds, nor does any special organic radical seem to be responsible for the characteristic test. As a group the alkaloids seem to give more of the positive test than does any other group."[10] (It is not quite clear, even in reading the whole article, whether, as they use the terms, either "the characteristic test" or "the positive test" means any strong color reaction, or whether only a "reddish violet" color.)

An examination of their article shows (a) That they list a considerable number of reactive phenols and ethers of phenols under other classes, including apomorphine, codeine, cryptopine, dionin, heroin, hydrastine, morphine, and narceine as alkaloids; tyrosine as an amino acid; arbutin as a glucoside; gallic and tannic acids as aromatic acids; and adrenalin under "miscellaneous aromatic compounds"; (b) That as nonreactive phenols they include seven nitrophenols which, it will be conceded, do not react with Fröhde's or similar reagents; and

(c) That as nonreactive phenols they also list some which, according to the observations on which this article is based, give very strong reactions; these include the cresols, monochlorophenols, phenol, orcinol, and thymol.

BUCKINGHAM'S REAGENT

This contains 10 to 15 gm. ammonium molybdate dissolved in 100 c.c. of pure concentrated sulfuric acid by heating on the water-path, and cooled before use. Buckingham originally used 1 gm. molybdate in 15 gm. H_2SO_4.[2, 3]

With morphine this reagent strikes a very dark purple, changing at once, with solution, to intense purplish red, even stronger, and also more persistent, than with Fröhde's reagent. After several minutes, this fades and changes to a weaker brown or olive brown. Also a deep blue color develops at the edge and gradually pervades the whole solution. This blue color is due primarily to the reagent and appears to some extent with the reagent alone, as it absorbs moisture from the air. The fine green color that develops with Fröhde's reagent is not produced by Buckingham's.

Thus, considering all the colors, the reaction with Buckingham's reagent is much inferior to that with Fröhde's. Since, however, the purplish red color is both stronger and more persistent with Buckingham's, it may be preferred in testing minute traces. The sensitivity may be conservatively set at 0.0004 mg. (0.4 gamma) with respect to this one characteristic color, using a single drop of reagent.

In any case, Buckingham's reagent is not a useless variation, since with some compounds other than morphine (salicylic acid and aspirin, for instance) the greater concentration of molybdate is necessary to the reaction.

CONCLUSIONS

1. Fröhde's reagent provides a sensitive and specific test for morphine, the main color sequence being intense purplish-red, changing to weak brown or even fading out completely, then developing as strong bright green.

2. Although the test has been known since 1866, it has nowhere hitherto* been adequately described, and most descriptions of the color sequence are incorrect. It does not seem possible to attribute the erroneous and varying descriptions given by eminent toxicologists to faulty observation; apparently some unknown impurity has often produced, in greater or less degree, a blue color which sometimes blends with and sometimes dominates the true colors of the reaction.

3. Besides erroneous descriptions of the colors, several mistakes and myths are very common. Many authors are confused in regard to the diference between Fröhde's reagent and Buckingham's variation. (Buckingham used a much higher concentration of molybdate.) Myths: that the reagent will not keep; that the colors are due merely to "lower oxides" of molybdenum.

4. In obtaining characteristic tests for reactive compounds, the "Fuming Fröhde's" (made with fuming H_2SO_4) and Buckingham's reagent are of great value, perhaps equally with the normal reagent.

*The writer's own description, without the discussion given here, has just been published as one of "The Principal Chemical Tests for Morphine," Am. J. Pharm. **109**: 228, 1937.

5. Compounds with which Fröhde's reagent reacts are, in general, phenols or ethers of phenols, or in some cases aromatic amines. All kinds of colors and sequences of colors are produced, and, in consequence, characteristic or specific tests can be obtained for many different compounds.

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CRYPT ASPIRATION: SPRAY CULTURE METHOD FOR THE ISOLATION OF B. DYSENTERIAE

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DURING the past few years we have been using the following method to advantage in our dysentery work. It is particularly useful in the chronic dysentery cases because recovery of the specific organism by ordinary routine fecal culture methods is usually unsuccessful.

Principle.—By means of a crypt aspirator and sigmoidoscope the material from the deep recesses of the crypts or ulcers is sucked out and sprayed directly upon Endo or other suitable plate medium.

Apparatus.—(1) Sigmoidoscope. Any type will do as long as it provides an adequately illuminated field for securing culture material by direct vision.

(2) Crypt aspirator. A 35 cm. heavy wall capillary glass tube (outside diameter 5.5 mm., bore approximately 0.5 mm., A. H. Thomas catalogue No. 6088) with the distal 2.5 cm. bent to an angle of approximately 20°. Just proximal to the point of angulation there is blown a small bulbous reservoir, sufficient to hold two or three drops of fluid. This is accomplished by sealing the distal end of the dry tube, heating the area to be expanded to a red heat

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and blowing through the proximal end until the desired size is obtained. With a little practice, angulation of the tip and bulbous reservoir can be made at one operation. The seal at the distal end is broken, and both ends of the tube smoothed off in the flame. An ordinary 20 c.c. rubber bulb, such as that which comes with therapeutic serum outfits, is used to create suction. The bulb has a

Fig. 1.—Mucosal aspirator for taking cultures from crypts or from floor of ulcer. Length of glass tube 35 cm. (14 inches), sufficient for insertion through a 12-inch sigmoidoscope. Note fusiform dilatation near tip which is bent slightly for better approximation to intestinal wall.

Fig. 2.—Plate inoculated by spray culture method. Note isolated colonies.

small hole in its dome, so that when it is attached to the proximal end of the aspiration tube it may be compressed without forcing air or fluid out of the tube. Then by placing the index finger over the hole, suction is created by releasing pressure on the bulb.

(3) Culture medium. We still find a properly prepared Endo plate satisfactory, but any suitable substitute may be found equally satisfactory.

Method.—The sigmoidoscope is passed with the patient either in the knee-chest position or on a proctoscopic table. The glass aspirator with bulb attached is passed through the open window until the angulated distal end is in direct contact with clean or nearly clean mucosa. A little mucus, blood, or pus, if present, may be aspirated together with the crypt contents into the small reservoir by first pressing the sides of the rubber bulb gently, and then placing the index finger on the dome hole, releasing the rubber bulb until it regains its normal shape. The index finger is removed. The aspirator is then withdrawn from the sigmoidoscope, the index finger again placed on the dome hole, and the contents of the reservoir sprayed on the Endo plate from a height of about 5 cm. Inasmuch as we boil the sigmoidoscope and tube before use, one or two drops of water remain in the capillary portion of the aspirator. Even if only sufficient material is aspirated from the intestinal crypts to fill up the mouth of the capillary bore, it is sprayed diffusely over the plate by the one or two drops of water left in the higher portion of the tube. Routine examinations for *E. histolytica*, nature of the cellular exudate and culture for *B. dysenteriae* can usually be made from a single aspiration. If necessary a drop or two of warm saline, broth, or sterile water may be expressed upon the mucosa before aspiration.

The spray method of culture may be used in isolating organisms from feces or mixed cultures in general bacteriologic work. A broth or saline suspension is sucked up into the reservoir and immediately expressed into the original container. A sufficient number of organisms adhere to the side of the reservoir, so that when the rubber bulb is compressed a second time over the culture dish a fine spray scatters the bacteria over the plate.

Since we have used sigmoidoscopic crypt aspiration and spray cultures, our percentage of positive cultures in chronic bacillary dysentery has risen from 13 per cent to almost 25 per cent. The explanation probably lies in the peculiar tendency for *B. dysenteriae* to lodge deep in the intestinal crypts in chronic cases. Our routine is to make a minimum of six sigmoidoscopic crypt cultures on different days before abandoning the search. Crypt material appears to be relatively free of the many contaminants present in fecal specimens. The spray method gives isolated, diffusely scattered colonies which can be easily picked off for further study.

THE CHEMICAL DIAGNOSIS OF PREGNANCY BY DETECTION OF ESTRIN IN URINE*

III. Modifications of Procedure and Results in 193 Determinations

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IN 1935 we described a method for the chemical diagnosis of pregnancy based on the detection of estrin in the urine,[1] and reported the results obtained with this method in 89 urinalyses. Since then we have simplified the procedure in several details of operation and apparatus, and we have done a total of 193 urinalyses. It is this further work which we wish to report at this time.

It may be well to briefly summarize the original method in order to understand the changes which have been made. The twenty-four-hour urine was brought to pH 4 with hydrochloric acid and evaporated down to about 200 c.c. The urine was then brought to pH 1 with concentrated hydrochloric acid and extracted for sixteen hours with ether in a special apparatus which consisted of a reflux condenser connected with four tubes, one containing the urine to be extracted, and three containing carbonate solution to wash the ether extract. (This apparatus has been greatly simplified.) The ether extract was then washed free of all aromatic hydroxy acids with sodium carbonate and, after removing the ether by distillation, the residue was distilled with water until free of all volatile phenols. The last traces of water were removed by drying in a glycerin bath, and the estrin was detected by coupling it with diazotized p.nitroaniline to form a deep colored azo dye. The amount of color was estimated as millimeters of standard solution of ferric chloride (33 per cent) necessary to match the color test placed at 10 mm., and this was called the F.N. (ferric chloride number), an F.N. over 25 indicating pregnancy.

APPARATUS

The principal modification has been the devising of a much simpler extraction-washing apparatus. Whereas our original apparatus required fairly skilled glass blowing in its construction and was difficult to assemble, the present device is assembled from the usual laboratory apparatus and requires but one T-sealed glass joint. The first extractor of this type was described by us[2] in April, 1936, and consisted of a urine extraction tube, a sodium carbonate washer, and an ether extraction flask. This has now been simplified

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even further, in that the carbonate washer has been eliminated by placing the sodium carbonate solution in the ether extraction flask. The final assembly is illustrated in Fig. 1.

Except for the sealing of the side arm to the Kjeldahl flask, which requires some experience,* the only other special apparatus is the glass inner tube. This may easily be made from ordinary 6 mm. soft glass tubing by blowing a bulb on the lower end. In order to provide a fine spray of ether, small holes are punched around the bulb with a strand of wire taken from a

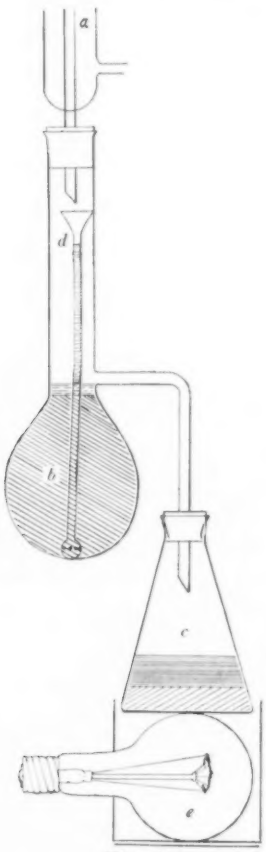

Fig. 1.

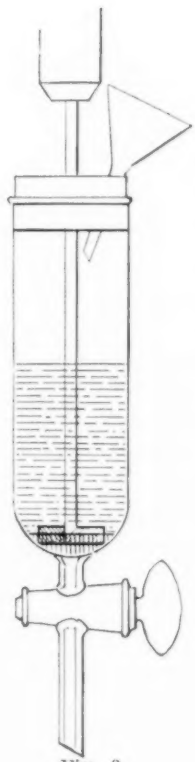

Fig. 2.

Fig. 1.—*a*, Condenser; *b*, 500 c.c. Kjeldahl flask to which is sealed a 11 mm. diameter sidearm as indicated; *c*, 250 c.c. Erlenmeyer flask; *d*, inner tube, 6 mm. diameter, with small holes at lower end; *e*, 100-watt lamp in asbestos lined tin can.

Fig. 2.—Apparatus for washing ether extract.

chromel wire gauze (A. H. Thomas Co., Catalogue No. 9993). This wire is sharpened to a fine point and held in a pliers or sealed into a glass rod. The bulb and pointed wire are heated with a fine pinpoint blow-torch flame, and with a little practice, fine holes are easily punched. The upper end of the inner tube is flanged out with a carbon rod from an arc lamp.

In addition to being more easily constructed, time is saved with this apparatus, since the urine need now be concentrated only to 400 c.c. instead of

*E. J. Callahan & Co., Baltimore, Md., can supply such flasks.

200 c.c. as formerly, and the apparatus is filled and assembled for operation much more quickly than the original one.

Before recommending the placing of carbonate solution below the ether in flask C (Fig. 1) experiments were done to determine that no estrin was lost in the carbonate. Full-term pregnancy urines were extracted, the carbonate underlayer was removed and washed twice with small portions of fresh ether which were then added to the main ether fraction. The carbonate was made strongly acid with hydrochloric acid and thoroughly extracted with ether. The ether residue was transferred to olive oil and injected into castrated rats. No estrin was demonstrable.

Our method of evaporating the twenty-four-hour specimen is of interest, since it has led to the construction of a simple device which automatically signals with a bell when the evaporation is completed. Since such an apparatus is of general interest in many operations where it is desirable to carry out odoriferous evaporations in distant hoods, we are describing it on page 642 of this issue. Using this apparatus, we have never lost a urine by accidental burning.

Another time-saving procedure has been developed in the method of washing the ether extract. At the time of our first report this was done by means of the usual separatory funnel. Much time and effort can be saved by washing in a cylindrical separatory funnel by means of a motor driven stirrer. This is illustrated in Fig. 2. The paddle should be placed just at the interface of the two phases, and the direction of rotation of the blades should be such that the carbonate is thrown up into the ether.

At the conclusion of the water distillation, the original procedure directed the removal of the last traces of water by heating in a glycerin bath at 160° C. This process was time-consuming and required frequent attention, for accidental elevation of temperature caused charring and seemed to diminish the red value given by positive tests, giving dark brown tints instead. This has been simplified by placing the distilling flask, containing the last 10-15 c.c. of water in a boiling water-bath and removing the water under vacuum; the ordinary laboratory water vacuum pump does this in a few minutes. The rubber hose from the pump is slipped directly over the side arm of the distillation flask.

PROCEDURE

The procedure is essentially the same as the original, but we should like to amplify more fully a few points which we have found to be sources of error to other workers when they first begin doing the test.

The pH of Hydrolysis.—At the time our paper[1] appeared it seemed likely from the work of others[3, 4] that an acidity for hydrolysis greater than pH 4 was necessary to obtain a maximum deconjugation of estrin esters. We pointed this out in a mimeographed supplement, the availability of which was noted at the end of our paper,[1] and we recommended therein the use of pH 1 as possibly giving better yields of free estrin and improving the results in the diagnosis of

early pregnancy. We noted that experiments to settle this point were in progress. The results of these experiments concerning the optimum acidity of hydrolysis have been reported in this journal.[5] Briefly summarized, they showed that after pH 4 hydrolysis and complete extraction of the deconjugated hormone, subsequent hydrolysis at higher acidities does release still more hormone, but the additional yield then obtained is always much lower than that first obtained at pH 4. On the other hand, with strong acid hydrolysis there is produced in urine a compound which is ether-soluble, insoluble in sodium carbonate from ether solution, nonvolatile with steam, and capable of coupling with diazotized p.nitroaniline to form a deep red azo dye; that is, it has all these chemical properties in common with estrin, but *it is not estrogenic*. This compound, which we are tentatively calling "chromogen," is produced in about 7 out of 10 urines when 15 per cent hydrochloric acid hydrolysis (15 c.c. of conc. HCl per 100 c.c. urine) is used and occasionally at pH 1, and is obtained from *any* urine. We have never obtained it at pH 4. While we do not deny that an acidity much greater than pH 4 may be necessary to quantitatively hydrolyze estrin esters, it is seen from this work that in this procedure such acidities may cause false positives due to the formation of chromogen. For accurate results in pregnancy diagnosis one must, therefore, use pH 4 hydrolysis as originally directed until the nature of the chromogen can be determined and a method for its removal devised.

After hydrolysis at pH 4, we suggested that the urine be adjusted to pH 1 before extraction, since there seemed to be evidence in the literature[6, 7] that extraction was more efficient at the latter concentration. From further work we now know that making the urine strongly acid at room temperature produces neither chromogen nor estrin deconjugation; boiling of the strongly acidified urine is necessary to obtain this. This is in agreement with the finding of Marrian[3] that high acidities at low temperatures produce no significant hydrolysis of estrin esters. The adjustment to pH 1 is therefore omitted.

The washing of the ether extract with sodium carbonate has been modified only in the fact that the saturated carbonate solution is prepared at the time of use, rather than preparing a large volume of stock solution as originally directed. The reason for this is that such a solution (sp. gr. 1.22 at 23° C.) will not remain saturated under the usual variations in laboratory temperature, especially in cold weather. It is simpler to stir an excess of carbonate with water just before use. We would stress at this point that this washing must be continued until the carbonate wash gives a negative test with 1 c.c. of the color reagent. This is best determined by comparison with a control made of an equal amount of carbonate solution and 1 c.c. of reagent. The two colors should be compared by looking through the length of the liquid column against a white background. Failure to wash completely will give an increased amount of false color in the final test. In the usual determination, 20 to 30 carbonate washes are required. We have observed that when the washing occasionally seems to progress very slowly that the interjection of acid washes seems to hasten the process. Acid washes are therefore used until they no longer show any change on testing with the reagent.

The acid washes (2 per cent HCl) are saturated with sodium chloride and are tested by adding 1 c.c. of color reagent and making alkaline with sodium carbonate solution. The acid washes and a final wash of distilled water always conclude the washing procedure; this is done because phenol is not volatile from strongly alkaline solution, and when ether is distilled off after prolonged washing with aqueous carbonate, there remains a fair amount of white residue, presumably carbonate.

The same precaution to insure completeness is to be stressed in the phenol distillation. Here, as above, the color given by distillate, 1 c.c. of color reagent and carbonate solution must match exactly a control made at the same time with distilled water. Incomplete distillation seems to be the most common source of error made by those first working with the procedure. The distillation is best conducted from a 500 c.c. distilling flask, the water level being maintained at between 150 to 300 c.c. The amount of distillate required before a truly negative test is obtained is usually about 700 to 1,000 c.c. Fresh corks in the distilling apparatus give markedly positive color tests in the distillate. This factor is completely eliminated by placing the delivery tube of the distilling flask well into the condenser without a cork and by closing the mouth of the flask with any loose fitting glass stopper; the condensed vapors seal it very efficiently.

No change has been made in the technique of the final color test nor the method of ferric chloride comparison. Many workers have told us that they experience difficulty in comparing the predominantly red color of strongly positive tests with the predominantly brown color of ferric chloride. This is quite true, and the comparison is more a balance of density rather than a true color matching; the ferric chloride does match the yellow-brown colors of negative tests. To obviate this difficulty, we have found that beta-naphthol solutions when coupled give a predominantly red dye which matches very well the red colors of positive tests. The beta-naphthol standard contains 0.2 mg. beta-naphthol in 8 c.c. of 95 per cent ethyl alcohol. This is made from 2 c.c. of a dilute stock standard, containing 0.1 mg. per c.c. by the addition of 6 c.c. of alcohol. To this is added 1 c.c. of the color reagent, and then 1 c.c. of 10 per cent sodium hydroxide. The reason for using sodium hydroxide here is that when beta-naphthol is coupled in sodium carbonate solution by the pregnancy test technique, it gives yellow orange rather than red colors. The BN (beta-naphthol number) is obtained exactly as the FN and can be converted to the FN by multiplying it by 5.97. The use of beta-naphthol is optional. While the 33 per cent ferric chloride solution reaches a color on standing which is quite stable for many months as checked by tintometer readings, beta-naphthol solutions decrease in tintometric value in several weeks.

In the interpretation of the final color test we have observed that the presence of a red tint which tends to stain the filter paper is very suggestive of increased estrin. Further experience would indicate that the FN interpretation as previously given, negative below 15, doubtful between 15 to 25, and

positive over 25, should be shifted to 20, 20 to 30, and over 30, respectively. While the limits of these readings apply to the vast majority of urines, one will occasionally encounter a negative urine, the final alcohol extract of which contains so much brown pigment (the color of which does not increase appreciably on coupling) that the FN will be about 25, but the test will be a pure brown without a trace of red. In most of the tests, however, no difficulty is encountered, since pregnancies as early as the fourth week often give an FN of 50 with markedly red color tests. In suspected earlier cases with an FN of 30, for example, one does not feel certain and prefers to do another test at a later date.

RESULTS

At this writing we have done 193 urinalyses by this method. The results are summarized in Table I.

TABLE I

	NO.	PER CENT
True positive results	99	51.3
True negative results	44	22.8
Doubtful results	19	9.9
False positive results	17	8.8
False negative results	7	3.6
Subsequent history could not be obtained	7	3.6
	193	100.0

Only those tests are designated as true positives or true negatives which subsequent clinical study over a period of months or longer corroborated. Almost all of the cases in our series, after the first few which we ran, were difficult problem cases; they all had gynecologic or obstetrical complications, some endocrinopathy, or a previous history which made the clinical diagnosis uncertain. The routine type of case, in which a pregnancy test is done because of a suggestive history and the absence of definite physical signs of pregnancy, was not accepted in this series. Doubtful results (results showing intermediate quantities of estrin excretion) obtained in conditions other than pregnancy are generally significant in that they are associated with some endocrine disturbance. In such cases, of course, later tests do not show the rising estrin excretion which is characteristic of pregnancy. Our 19 doubtful tests included the following conditions:

4 Threatened abortion.
3 Less than four weeks pregnant; confirmed by later tests.
3 Teratoma testis.
2 Irregular menses and pelvic mass.
1 Missed abortion.
1 Fetal skeleton in abdominal cavity for three years.
1 Eleven to twelve weeks pregnant.
1 Ten days postpartum.
1 Menses delayed two weeks—early abortion (?).
1 Contracted pelvis: three to four months pregnant, 2 Friedman tests negative, estrin bioassay doubtful.
1 Marked obesity; six months pregnant.

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Of our 17 false positive results, a few may have been due to technical errors, but, on the whole, they probably represent conditions in which for pathologic reasons at present unknown there is actually an increased estrinuria. They were distributed as follows:

4 Theelin administered in large dosage before specimen was collected.
5 Amenorrhea—origin undetermined, clinically not pregnant.
1 Six-week menstrual cycle. This specimen measured 4,760 c.c. (48 hours ?) and was passed just before the menses (normal peak of urinary estrin).
1 Ruptured ectopic pregnancy.
1 Nine-year-old female—endocrinologist's examination in hospital history quoted: "Hypertrichosis—pubic and general . . . breasts well developed and large areola . . . she has certain signs and symptoms suggestive of hyperadrenalism . . . premature appearance of secondary sex characteristics. . . Possibly basophilic adenoma of pituitary and secondary hyperplasia of adrenal cortex."
1 Pituitary dysfunction, diabetes insipidus, multiple xanthomatosis, Hand-Schuller-Christian disease (?). Fourteen-year-old female, amenorrhea, obesity, polydipsia, polyuria.
1 Hypopituitarism (?). Twenty-seven-year-old female, abnormal hair distribution, occipital headaches, irregular menses, laparotomy—adrenals normal, ovary prolapsed.
1 Uterine fibroid.
2 One patient, middle-aged male. Testicular swelling decreasing in size on x-ray irradiation; followed by operative removal. Pathologic diagnosis—inflammatory disease. Clinical diagnosis—teratoma testis.

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The case listed above of possible hyperadrenalism is of interest since Saphir and Parker[8] have also reported increased urinary estrin in a case of hyperadrenalism.

False negative results were obtained in the following 7 analyses:

1 Four days past expected menstrual period—ten days later gave positive test.
1 Three weeks past expected menses; Friedman test positive.
1 Pregnancy with marked obesity; also gave 1 doubtful test.
1 Clinically eight weeks pregnant; at four weeks gave positive test.
3 One patient—constantly denied sexual intercourse; pregnancy confirmed by x-ray.

7

The last case listed gave 3 negative tests at about the second, third, and fourth months of pregnancy. She vigorously denied any possibility of being pregnant and desired operative interference. Pregnancy was verified at about the fifth month by x-ray. (We believe that any patient capable of such persistent prevarication is also quite apt to substitute urine of some other person in her efforts to obtain operative intervention.) Crew,[9, 10] in a very wide experience with Aschheim-Zondek and Friedman tests, explains somewhat similar results as due to psychic inhibition of hormone excretion.

The conditions listed above which gave false positive or doubtful results did not do so uniformly. Correct results were obtained in cases of teratoma testis, ovarian cyst, fibroids, missed abortions, diabetes insipidus, ectopic pregnancy, and "endocrinopathies"—including pituitary dyscrasias. We have observed in several cases that when bleeding complicates pregnancy, the urinary estrin is much lower than normal.

DISCUSSION OF RESULTS

While an incidence of 8.8 per cent of false positive results may seem high in comparison with the Friedman test, which is commonly stated to give an accuracy of 98 per cent, we would point out that when prolan tests are done on a series of *problem* cases such as this, the incidence of false positive results is just as high or higher. This fact has been demonstrated by many workers[11-15] and, we feel, it is not sufficiently well recognized by those who write the usual textbook figures of 98 per cent accuracy for Friedman tests without any qualification.

In our present series of cases, one patient, two months pregnant, gave a negative Friedman test and positive chemical test. Another in which the date of intercourse was known, gave a negative Friedman and positive chemical test at three and a half weeks of pregnancy; the Friedman test repeated on the same rabbits at the seventh week was positive. While our number of such cases is certainly too small to say that the chemical test is as reliable as the Friedman test in early pregnancy, this may be the case. In this connection it should be pointed out that Browne and Venning[16] and Evans and his associates[17] have recently upset the hitherto long accepted belief in a very high prolan excretion in the "first weeks" of pregnancy by demonstrating "the invariable existence of an exceedingly steep and high hormone peak at a time which is quite accurately one month from the beginning of the first expected but missed menstruation" (quoting Evans). It is possible that false negative prolan tests in early pregnancy are to be explained by this peak not yet having been reached.

One of the great advantages of chemical estimation of estrin by the colorimetric method, as contrasted to the biological pregnancy tests, is that a relatively quantitative estimation of hormone excretion is obtained. This quality of the procedure has been utilized by Savage and Wylie[18] in studying the differences in estrin excretion in normal pregnancy, preeclampsia, and chronic nephritis complicating pregnancy.

OTHER CHEMICAL REACTIONS FOR ESTRIN

We have made many attempts to shorten or modify our procedure, or to find some other reaction which could be carried out very rapidly, but so far these efforts have been without success.

The meta-dinitrobenzene reaction[19] of theelin, the silico-tungstic acid reaction,[20] and the concentrated sulfuric acid reaction[21] of theelin and theelol in our experiments have not been sufficiently sensitive to react with quantities of hormone demonstrable with the diazonium reagent. The trichloracetic acid reaction, and the concentrated sulfuric acid—acetic anhydride reaction of sterols gave negative results with quantities of hormone extracts which gave strongly positive color reactions with the diazonium reagent.

The simple chemical pregnancy test of Visscher and Bowman[22] (not a test for estrin) has not shown in our hands any difference in reaction between normal male urines and those of advanced pregnancy.

We have attempted to eliminate the process of ether extraction by evaporating the urine to dryness and leaching the residue with ether but could obtain no estrin in this manner, although equal fractions of the same urine gave positive reactions by our routine procedure and by extraction and bioassay.

The Marrian method of tintometric estimation[23] of theelin and theelol with phenolsulfonic acid has given us good results with late pregnancy urine; we have thus far found it unsatisfactory for demonstrating estrin in early pregnancy.

With the appearance of the tungstic acid method of precipitating urinary estrin,[24] we thought at first that this method might eliminate our long extraction of estrin with ether. However, our results with this method of extraction have uniformly given excessively high amounts of color on normal male urines.

We have recently found that norite (activated charcoal) quantitatively adsorbs estrin from pregnancy urine. Conjugated estrin seems to be adsorbed as well as free estrin. Several experiments indicate a very efficient elution of the norite adsorbed estrin by butyl alcohol. The much smaller bulk of norite precipitate and the simplicity of this elution as contrasted to the elution in the tungstic acid method make this method worthy of further study in an effort to simplify the rapid removal of estrin from urine.

SUMMARY

Modifications and improvements in a method for the chemical detection of urinary estrin are described. Results obtained with this method in 193 determinations made mostly on problem cases are presented and discussed. Results obtained in attempts to diagnose pregnancy by other rapid chemical methods are given.

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AN AUTOMATIC EVAPORATION CONTROLLER AND SIGNAL*

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ALTHOUGH open evaporation by heat is a useful laboratory practice, the procedure involves repeated inspections in order to control the degree of concentration, or to prevent damage to some valuable biological fluid or organic solution. This supervision is tiresome and time-consuming, especially if the evaporation is going on at some distance from the operator. Losses often occur, both of material and apparatus, because of forgetfulness, procrastination or interference.

After a few discouraging experiences with urine evaporations coincident to hydrolysis of estrin-esters for use in a chemical test for pregnancy,[1] the author devised a simple apparatus which gives a bell signal when the evaporating liquid reaches any predetermined volume or level.

Because the device has proved to be reliable over a period of two years, a brief description is given in the belief that it may be of value to other workers.

Description.—Fig. 1 shows the front view of the apparatus when clamped in operative position. Fig. 2 shows the top plan, with the electric contact assembly swung 180° out of its usual position on *D* in order to illustrate the shapes of the trunnion arms *I-I* which support *J* and *K*, Fig. 1. All metal parts are of brass. The figures are not to scale.

*From the Department of Biological Chemistry, University of Maryland, School of Medicine.

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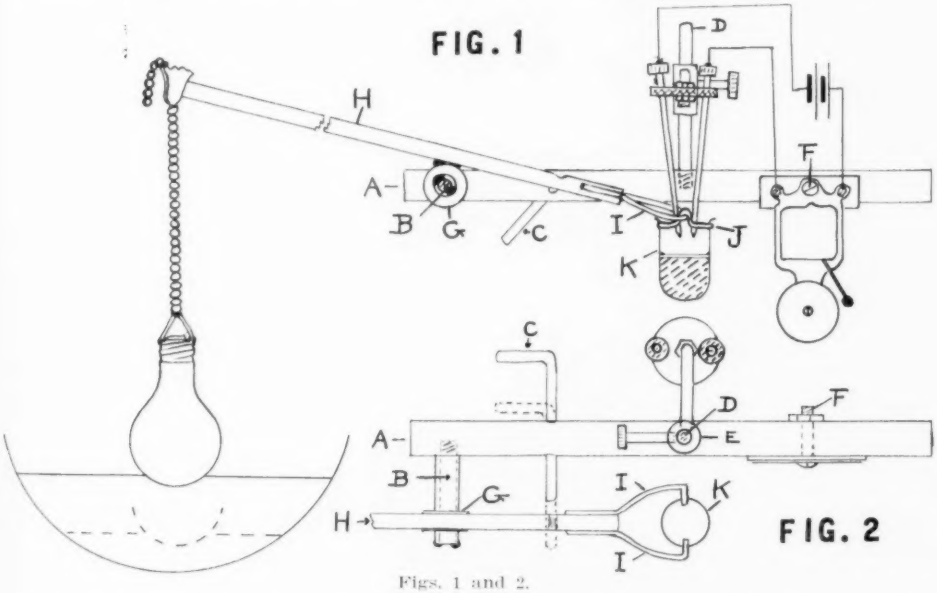
A is a 12-inch piece ½-inch rod; *B* is 3/16-inch rod, 2¼ inches long, which is threaded or sweated in *A* at ½ inch from its end; *C* is ⅛-inch rod, 4½ inches long, bent as shown, and fits loosely in a hole drilled through *A* at 2½ inches from *B*, and parallel to *B*; *D* is 3/16-inch rod, 3¾ inches long, and is threaded into *A* at 5½ inches from *B* and at right angles to *B*. *D* supports the electrical contact assembly through a heavy collar *E*, Fig. 2. The latter slides up and down on *D* and is fitted with an adjusting set-screw and a 3/16-inch support. This support is bent and threaded for clamping the 1¼-inch circular or square insulating block, ¼ inch thick, in which the two converging contacts or electrodes are held tightly by friction. The electrodes, 4 inches over all, are made of ⅛-inch rod. They are fitted with knurled nuts at the top and are pointed at the lower ends which center on *K* when in place. *F* is a bolt through *A* which clamps an electric bell in place against a piece of flat insulating fiber.



Figs. 1 and 2.

Returning to the left end again, *G* is a washer ½ inch in diameter, ¼ inch thick, and with a 3/16-inch hole. It is fitted to rotate freely on *B*. *G* is held loosely in place by collars on *B*, the outer shorter collar is soldered to the free end of *B*. *G* is spaced 1½ inches from *A*. *H* is a piece of 3/16-inch rod, 12 to 16 inches long. It is soldered to *G* at a point 4 inches from its right end as shown in Fig. 1. *I-I* are made of 1/16-inch wire, soldered to *H* as shown in Fig. 2, and shaped to act as trunnions which suspend the collar *J* at 1½ inches from the right end of *H* (Fig. 2). *J* is a collar made of 1/16-inch wire. It is formed to suspend counterweight tube *K* which is made from the lower 1½ inches of a 1-inch diameter test tube (or a small beaker). *K* is flared at the outlet to retain *J* in position; *K* should swing freely in its fittings.

On the left end of *H* a split bell from a pull-chain is soldered in an inverted position, with the slit away from the end of *H*. Into the bell is adjusted the desired length (12 to 16 inches) of pull-chain. To the lower free end of this chain the apex of a v-shaped piece of 1/16-inch wire is soldered, which in turn is soldered as shown to the base of a 25-watt frosted-inside electric bulb.

The whole apparatus may be of simpler design and yet be useful. For example, *A* may be a piece of wood, provided with a metal extension for clamping to a stand. *B* and *D* may be nails, and *G* a brass battery nut. Ordinary insulated wires may be wound around *D* forming electrodes at their free ends. *H* may be a cheap curtain rod, split at the right end to encircle and support *K*, thus omitting *I-I* and *J*. The bell is screwed to the wood body of the apparatus. This simple arrangement may be assembled from materials in any laboratory. In fact, our first controller was built in this way and is still dependable.

Operation.—Clamp the right end of *A* to a laboratory stand. Wire the bell and electrodes in series with two 6-inch dry cells, as shown. (The bell may be wired to the operator's laboratory if more convenient. In this case use three dry cells.) *Do not use a switch.*

With the float bulb hanging freely, and stop *C* pulled out of the way, add clean metallic mercury to counterweight tube *K*, until the bulb is in balance. Add a $\frac{1}{16}$-inch layer of clean mineral oil to the surface of the mercury.

Place two or three pieces of chromel wire gauze on a large tripod. This is important for heat distribution from a large flame, as from a quadruple burner.

Prepare an apron, 16 inches square of 4 to 6 thicknesses of asbestos paper clamped together by a paper-stapling machine. Cut a 4- to 6-inch center opening and place this over the tripod. (This apron prevents charring of the contents of the receding liquid as it drys on the walls of the container.)

Now raise the float beam *H* at the left end and hold here by stop *C*.

Place a porcelain dish on the prepared tripod and press it firmly into place. Measure into the dish the predetermined volume at which you wish the apparatus to give a signal. Now release the beam and adjust the weight of mercury in the counterweight tube and the length of the suspension chain, so that the rounded portion of the bulb will sink about $\frac{3}{8}$-inch below the surface at this level.

Adjust the electrodes coarsely with the sliding collar, and delicately by rotating one electrode in the insulating block, so that the bell rings at this point. Pour in the remainder of the liquid; this raises the bulb. The bell stops ringing. Raise the bulb again about 3 inches. It should fall to the surface at the higher liquid-level from this point.

Light the burner and forget the evaporation—until the bell rings again.

Advantages.—This simple controller has been used to control hundreds of evaporations without a single loss of apparatus or material.

The electrical contacts are dependable; they are sensitive and do not corrode.

The apparatus may be used to control the evaporation of very large or small quantities of liquids.

With the large 30-liter dishes the controller is effective down to a level where the final liquid depth is about 20 mm.

Using smaller porcelain dishes, it is possible to evaporate 4,000 c.c. to 100 c.c., or by using a 200 mm. dish, to evaporate 300 to 400 c.c. to 25 c.c. with safety.

NOTE.—The author has devised a more complicated apparatus which will begin to turn down the gas flame at any desired stage of the evaporation, then will simultaneously turn off the gas entirely and give a bell signal at its completion. The author will be glad to send line drawings of this more complex apparatus to interested investigators.

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A SIMPLE PROTECTION IN ASEPTIC GRINDING OF TISSUE

FLORENCE L. EVANS, PH.D., CARVILLE, LA.

WHEN grinding tissue in a mortar, it has been found that the following device aids materially in promoting asepsis, as well as protecting the operator from splashed droplets or bits of sand.

This device consists of an apron of paper toweling, cloth or gauze, fitted loosely over the mortar and tied firmly to the pestle with string. This apron

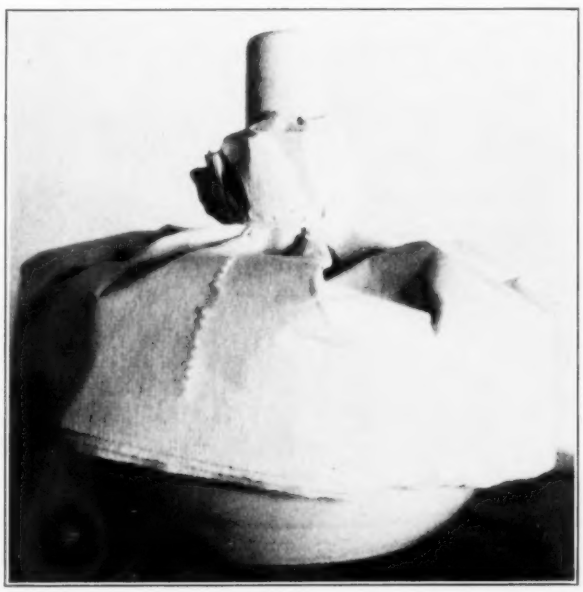

Fig. 1.

is made of a straight piece of paper or other material about one and a half times the circumference of the mortar, and long enough to come well over the edge of the mortar. It is fastened to the pestle by gathering it in and tying with a piece of string. The apparatus with the apron in place is wrapped and sterilized as usual. At the time of use, the edge of the paper can be folded up. This forms an umbrella-like shield which protects the contents of the mortar from external contamination during grinding.

From the Laboratory of the U. S. Marine Hospital, Carville.
Received for publication, August 25, 1937.

A TIMESAVING METHOD FOR EXAMINING BLOOD FILMS WITH THE HIGH DRY (45x) AND OIL IMMERSION OBJECTIVES*

FLORENCE L. EVANS, PH.D., CARVILLE, LA.

THE advantage of this method is that a large surface of the slide can be examined in less time than with the oil immersion objective. The effect is as brilliant as though the smear were permanently mounted under a cover glass.

The method consists of spreading a thin even film of immersion oil over the entire slide. A straight piece of glass rod or tubing is best for spreading the oil. If it is uneven, permitting the slide to stand on the desk a few minutes will remedy this. The film of oil should be thin enough to permit focusing

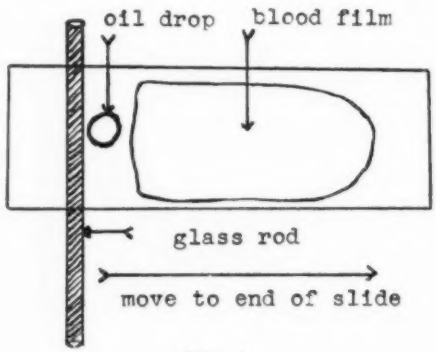

Fig. 1.

with the high dry lens without getting oil on it. Doubtful or interesting cells can be studied under the oil immersion objective simply by rotating the nosepiece.

FURTHER OBSERVATIONS ON THE USE OF NIGROSINE TO DEMONSTRATE TREPONEMA PALLIDUM IN SYPHILITIC LESIONS†

ROBERT B. DIENST, PH.D., AUGUSTA, GA.

THE detection of *T. pallidum* in lesions is of great diagnostic value in doubtful cases of syphilis and a factor of considerable importance in the relation to an early diagnosis.

A method for the demonstration of *T. pallidum* in syphilitic lesions was recently described by Dienst and Sanderson. *Treponema pallidum* is difficult to stain with the dyes ordinarily used in bacteriologic examination, consequently its characteristic morphology in exudates can best be demonstrated by dark-

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field illumination or by some method which stains the background and leaves the spirochetes and other organisms colorless. Nigrosine was found to be far superior to any other agent used for this particular purpose.

PREPARATION OF STAIN

The stain is prepared by dissolving 5 grams of nigrosine (Coleman and Bell) in 100 c.c. of distilled water. This is accomplished more easily by heating the mixture in an Erlenmeyer flask in boiling water for thirty minutes. Heating in the autoclave at 15 pounds pressure for fifteen minutes is equally satisfactory. One-half a cubic centimeter of 37 per cent formalin is added as a preservative. The stain should be filtered before being used.

METHOD OF STAINING

Use the ordinary bacteriologic inoculating needle and take one small loopful of the exudate from the freshly cleansed lesion and place it in a small loopful

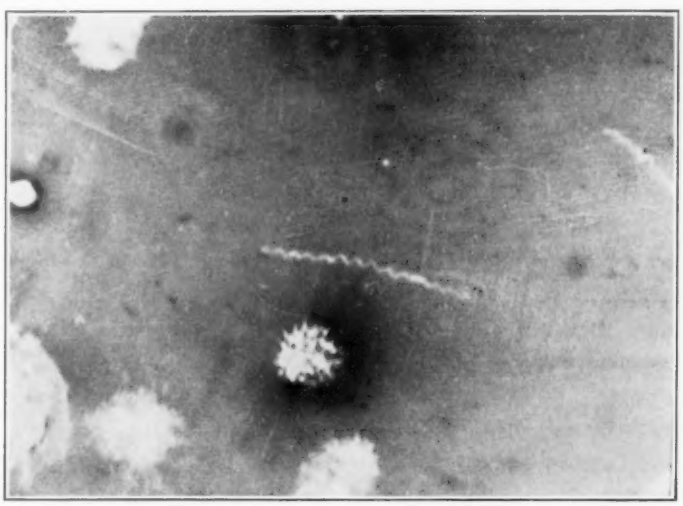

Fig. 1.—*T. pallidum* from a syphilitic lesion as demonstrated by the use of nigrosine (×2500).

of water on a clean slide. Then add a loopful of 5 per cent nigrosine and mix rapidly by rotary movements; then gradually increase the size of the smear by spreading out from the center. Satisfactory thinness of the preparation is obtained in this manner. Place a drop of immersion oil directly on the smear and examine for spirochetes, using a good substage lamp for sufficient lighting. The *T. pallidum* if present will appear as finely coiled, colorless organisms, and the remainder of the area will be stained gray to blue black, depending on the thickness of the preparation. A very sharp focus is necessary to visualize the spirochete of syphilis because of the delicateness of its structure (Fig. 1). When the preparation is properly made, the delicate terminal filaments of the spirochete are sometimes visible by this method.

Very satisfactory results have been obtained from exudative material after it has dried on the slide. In this case a loopful of water is placed on the area of the dried exudate and the material dissolved. Then a loopful of the dissolved exudate is placed on a clean slide and a loopful of 5 per cent nigrosine is added. Smear, dry, and examine in the same manner as for the fresh exudate.

RESULTS

Exudates were examined from 50 patients with the following results: Thirty-six patients were diagnosed positive either by the dark-field illumination test or by serologic blood tests, and thirty-five exudates from these same thirty-six patients were found to contain *T. pallidum* by the nigrosine preparation. One particular specimen was diagnosed positive by the dark-field test, but the spirochetes seen in the nigrosine preparation were not morphologically typical of *T. pallidum*.

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THE DETERMINATION OF SULFANILAMIDE IN CEREBROSPINAL FLUID BY MEANS OF SODIUM-BETA-NAPHTHOQUINONE-4-SULFONATE*

E. G. Schmidt, Ph.D., Baltimore, Md.

RECENT experimental and clinical studies with sulfanilamide show that this drug is a very powerful chemotherapeutic anti-infective agent.[1-5] Marshall and his associates have devised very good methods for the determination of the sulfanilamide content of alcoholic[6] and toluenesulfonic acid[7] filtrates of blood and other body fluids. In an extended investigation of the reaction between sodium-beta-naphthoquinone-4-sulfonate and various amino compounds,[8] information was secured which led to the development of a quantitative method for the determination of the sulfanilamide content of tungstic acid blood filtrates.[9] A direct method for cerebrospinal fluid analysis is now offered which utilizes the same reagent but eliminates filtrate preparation.

EXPERIMENTAL

The spinal fluids were obtained from the University Hospital laboratory through the kindness and cooperation of Miss B. Jahns. Although cerebrospinal fluid contains many compounds, such as amino acids, amines, ammonia, etc., which react with the beta-naphthoquinonesulfonic acid in slightly alkaline solution, no reaction or color intensification occurs with this reagent if the spinal fluid is first made slightly acid. In our previous work,[8] however, it was found that certain aromatic compounds like sulfanilamide, sulfanilic acid, anisidine, aminophenol, etc., gave colored solutions or precipitates with the chromogenic reagent even in acid medium. This fact permits the determination of sulfanilamide in blood and cerebrospinal fluid despite the presence of compounds which normally react with the quinone reagent.

*From the Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore.

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One cubic centimeter of spinal fluid is diluted to 10 c.c. with distilled water. Ten cubic centimeter quantities of various standards containing 0.01 to 0.20 mg. sulfanilamide, equivalent to 1 to 20 mg. per cent fluids, are also measured into test tubes. After the addition of 1 drop of 1.5 N HCl, the pH of each solution will range from 2.0 to 2.5. This degree of acidity prevents any reaction between the reagent and the nitrogenous constituents normally present in the spinal fluid. One cubic centimeter of freshly prepared 0.05 per cent sodium-beta-naphthoquinone-4-sulfonate (Eastman No. 1372) is added to each tube,

TABLE I

THE RECOVERY OF SULFANILAMIDE ADDED TO CEREBROSPINAL FLUID BY THE BETA-NAPHTHOQUINONE-SULFONIC ACID AND MARSHALL DIAZOTIZATION METHODS

SULFANILAMIDE ADDED	SULFANILAMIDE FOUND BY THE BETA-NAPHTHOQUINONE-SULFONIC ACID METHOD	ERROR	SULFANILAMIDE FOUND BY MARSHALL'S METHOD	ERROR
mg./100 c.c.	mg./100 c.c.	per cent	mg./100 c.c.	per cent
20.0	20.41	+2.05	20.51	+2.55
15.0	15.23	+1.53	15.46	+3.06
10.0	9.85	−1.50	9.80	−2.00
7.5	7.35	−2.00	7.58	+1.07
5.0	5.00	0.00	4.95	−1.00
2.5	2.51	+0.40	2.48	−0.80
1.0	1.05	+5.00	0.96	−4.00

their contents mixed and placed in the dark for one hour. The unknown is now read in the colorimeter against the standard which it most closely matches. The orange red color given by the reaction products shows poor proportionality, hence the standard and unknown must be of approximately the same concentration. It is generally necessary to prepare 2, 4, 7, 10, 15, and 20 mg. per cent standards. After the colors have been developed, any two standards may be mixed should an intermediate standard be required to secure a match with the unknown.

TABLE II

COMPARATIVE ANALYSIS OF THE CEREBROSPINAL FLUID OF PATIENTS UNDERGOING SULFANILAMIDE THERAPY

EXP. NO.	BETA-NAPHTHOQUINONE-SULFONIC ACID METHOD	MARSHALL'S METHOD	DIFFERENCE	CLINICAL DIAGNOSIS
1	15.1*	14.70*	−0.40*	Meningococcus meningitis
2	15.0	15.4	+0.4	Meningococcus meningitis
3	10.0	9.74	−0.26	Meningococcus meningitis
4	7.62	7.70	+0.08	Streptococcus viridans meningitis
5	6.96	7.4	+0.44	Streptococcus viridans meningitis
6	2.35	1.82	−0.53	Streptococcus viridans meningitis

*The values are given as mg. sulfanilamide per 100 c.c. of cerebrospinal fluid.

Measured quantities of sulfanilamide added to spinal fluid were fully and accurately recovered. Thus 1 c.c. of stock solution containing 2 mg. of sulfanilamide was added to 9 c.c. of pooled spinal fluid, yielding a 20 mg. per cent fluid. In a similar manner spinal fluids of various sulfanilamide content were prepared and analyzed as outlined above. In addition 3 c.c. quantities of each of the sulfanilamide-containing spinal fluids were treated with 27 c.c.

of alcohol, and the corresponding filtrates subjected to analysis according to Marshall's original procedure.[6] The data in Table I reveal the fact that excellent sulfanilamide recovery can be secured by both methods. Cerebrospinal fluids secured from patients undergoing sulfanilamide therapy were likewise subjected to analysis. The data in Table II show that both methods yield values which check closely with each other. Marshall's method is characterized by greater color intensity and better proportionality, the naphthoquinone-sulfonic acid method by greater simplicity.

SUMMARY

1. A simple method employing sodium-beta-naphthoquinone-4-sulfonate has been developed which accurately determines the sulfanilamide content of cerebrospinal fluid. Filtrate preparation is not required.

2. The values given by this method on spinal fluids secured from patients undergoing sulfanilamide therapy were found to check closely with the values given by Marshall's diazotization method.

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THE DETERMINATION OF PARA-AMINOBENZENE SULFONAMIDE (SULFANILAMIDE) IN URINE AND BLOOD*

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THE increasingly widespread use of sulfanilamide suggested the probability that various materials might be submitted to the public health and clinical laboratory for examination and determination of sulfanilamide content. A satisfactory method for such determinations was therefore sought. This investigation was begun before the deaths were reported from the so-called "Elixir of Sulfanilamide."[1]

The general method of Marshall, Emerson, and Cutting[2] was adopted. This method involves diazotization of the amino group of sulfanilamide by nitrous acid and its subsequent coupling with dimethyl-alphanaphthylamine to form a colored compound. The various concentrations of this compound may be readily determined by colorimetric comparison with standard solutions of sulfanilamide similarly treated. Several compounds other than dimethyl-alphanaphthylamine, however, were tried for coupling with the diazotized sulfanilamide.

The following compounds, because of their chemical structure, seemed to offer the best possibilities, and the results are as follows:

(1) *Dimethylaniline.*—Good gradation of orange color developed in ten to fifteen minutes. Blank lemon yellow.

(2) *"H" Acid (1-Naphthol-3, 6-Disulfonic Acid, 8-Amino).*—Good gradation of color running from orange to red with increasing concentration of sulfanilamide, developed in fifteen minutes. Blank yellow.

(3) *Amino "G" Acid (2-Naphthylamine-6, 8-Disulfonic Acid).*—Of no value, color slow in developing, difficult to compare.

(4) *Beta-naphthylaminesulfonic Acid "F" (2-Naphthylamine-7-Sulfonic Acid).*—No value, golden yellow in blank as well as in standard solutions of sulfanilamide.

(5) *Amido "R" Acid (2-Naphthylamine, 3-6 Disulfonic Acid).*—No value, pale yellow color only after long-standing.

(6) *Metaphenylenediamine.*—No value.

(7) *Diphenylamine.*—Good color gradation from colorless in blank to golden yellow of increasing intensity with increasing concentration of sulfanilamide. Color best after ten to fifteen minutes. This substance (diphenylamine), on the basis of the consistent results obtained, was adopted as the standard reagent for making the determinations for sulfanilamide. A difference in concentration of 0.1 mg. sulfanilamide per 100 c.c. of solution could be readily differentiated.

*From the Department of Public Health, San Francisco.
Received for publication, Jan. 21, 1938.

The method used by Marshall, Emerson, and Cutting[2] will determine the sulfanilamide in any combination to which it may be changed within the human body, so long as the amino group is intact and free, or in which the amino group may be rendered free quantitatively by hydrolysis before determination. This holds true with the modified method as here presented.

The method suggested in this paper is as follows:

Reagents.—0.1 N hydrochloric acid.

0.1 per cent aqueous solution of sodium nitrite (freshly prepared).

0.5 per cent solution of diphenylamine in 95 per cent alcohol.

Standard solution of 200 mg. sulfanilamide per liter of water, from which suitable standards for comparison may be prepared.

95 per cent alcohol.

Urine.—Dilute about 1:50, to contain approximately 0.5 to 1.5 mg. sulfanilamide per 100 c.c. To 10.0 c.c. of diluted urine, add 2.0 c.c. of 0.1 N HCl, 1.0 c.c. sodium nitrite solution, and 5.0 c.c. of alcohol in turn with shaking. After three to five minutes, add 1.0 c.c. of the diphenylamine solution. Compare with standard sulfanilamide solutions similarly treated. The comparison is best made about fifteen minutes after adding the diphenylamine.

Marshall, Emerson, and Cutting[2] have shown that sulfanilamide is partly excreted in a conjugated form (largely para-acetylaminobenzene sulfonamide) which may be determined only after hydrolysis to free the amino group. This hydrolysis is accomplished by heating for thirty minutes in boiling water equal volumes of urine and normal hydrochloric acid. The solution is then cooled, one drop of phenolphthalein solution added, and neutralized with 2 normal sodium hydroxide. The resultant solution is then diluted and treated as above.

The method of Marshall and his associates, as here modified, was found to give consistent results on human urine following administration of sulfanilamide. As indicated by determinations before and after hydrolysis, as much as 40 per cent of the sulfanilamide may be excreted in the conjugated form.

Blood.—The method of Marshall and his coworkers was used for determining free sulfanilamide in the blood, except for the use of diphenylamine to produce color. One volume of blood is run slowly with shaking into 9 volumes alcohol, stoppered, and let stand fifteen minutes. The mixture is filtered and 10.0 c.c. of filtrate measured into a small flask. Five cubic centimeters of water, 2.0 c.c. of 0.1 N HCl, and 1.0 c.c. of sodium nitrite solution are added with shaking. After three to five minutes, 1.0 c.c. of the diphenylamine solution is added. The colored solution is turbid, but may be filtered after about five minutes, and the filtrate used for colorimetric comparison. Standards are prepared by adding 1.0 c.c. of sulfanilamide solution of suitable strength to 9.0 c.c. of alcohol and treating this solution in the same way as the blood filtrate. The color is best about fifteen minutes after addition of the diphenylamine.

For the determination of hydrolyzable conjugated compounds of sulfanilamide in the blood, the following method has yielded the best experimental results:

To 10.0 c.c. of the blood filtrate as prepared above, add 5.0 c.c. of 0.1 N HCl, heat in boiling water for thirty minutes with reflux condenser. The solution is cooled and treated as previously described, omitting the addition of 5.0 c.c. of water and the hydrochloric acid. Standards are prepared as before, except that no water is added, and 5 c.c. of 0.1 N HCl are added in place of 2 c.c. However, use of the standards as prepared for determination of the free sulfanilamide in blood will apparently produce an error of not more than 10 per cent.

In order to determine whether or not some of the commonly used drugs which contain amino groups might produce color which could be mistaken for an indication of the presence of sulfanilamide in urine, urine was collected after ingestion of acetanilide and after acetphenetidin. Neither sample produced color under the conditions of the determination, either before or after hydrolysis with hydrochloric acid.

SUMMARY

1. The method presented for hydrolyzing conjugated compounds of sulfanilamide in the blood is perhaps of more value than is the change from dimethyl-alphanaphthylamine to diphenylamine in the determination.

2. It is found that as much as 40 per cent of the sulfanilamide excreted in the urine may be in a conjugated form.

3. Two common drugs, acetanilide and acetphenetidin, are shown not to interfere with determination of sulfanilamide in urine.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

WEIL'S DISEASE, Report of Seven Cases, Gaines, A. R., and Johnson, R. P. Arch. Int. Med. **60**: 817, 1937.

The diagnosing of Weil's disease in seven cases, six of them being noted within a period of six months, indicates that this condition may not be rare.

Because of the fact that five showed an atypical course, according to the standard textbook picture, the authors believe that this disease should be considered more often when there is unexplained jaundice.

The source and mode of infection in these cases have not been determined. This is true not only for this series but also for the previously reported cases in North America, except for one instance of accidental laboratory infection.

TRANSFUSION, Reaction Following Intra Group, Culbertson, C. G., and Ratcliffe, A. B. Am. J. M. Sc. **192**: 456, 1937.

The relation of anomalous agglutinins to transfusion has been an unsettled question. It might be questioned that the agglutinin was responsible for the reaction in this case. However, the facts may be summarized as follows: a Group 0 patient was given 500 c.c. of Group 0 blood; the cells of this blood were susceptible to agglutination by an anomalous agglutinin of low titer existing in the serum of the recipient. The recipient exhibited the typical symptoms of a delayed transfusion reaction, followed a typical course, and eventually recovered. A second patient, exhibiting a similar anomalous agglutinin, was given 550 c.c. of blood compatible with her intragroup agglutinin, and no sign of reaction was observed. Until the contrary is shown, the authors shall continue to regard incompatibility resulting from anomalous agglutinins as a definite contraindication to transfusion with such a blood.

In each of the 2 cases the low-titer intragroup agglutinin was unobserved in the routine compatibility tests by the hanging-drop method, although it was readily demonstrated by the centrifuge test method.

They suggest the use of the centrifuge test method given below as an aid in avoiding transfusion reactions of the type mentioned above.

Technique for the Centrifuge Test Method:

1. Place 3 drops of serum in a small test tube (14 mm. diameter).
2. Add 1 drop of cell suspension (3 drops of blood from a 20-gauge needle in 4 c.c. of a physiologic saline solution containing 0.2 per cent sodium citrate).
3. Shake to mix. (A control of saline and cells is optional after a little experience.)
4. Incubate five minutes at 37° C.
5. Centrifuge three minutes at high speed.
6. Note any evidence of hemolysis.
7. Shake the tubes to resuspend the cells while observing them with a concave mirror or hand lens. Note any evidence of clumping.
8. Pour the fluid onto a glass slide and examine microscopically for agglutination.

BLOOD GROUP, Relation of, to Blood Diseases and Neoplasms, MacLeod, K. I. E. Brit. M. J., p. 745, October 16, 1937.

Before any conclusions can be formed as to the relation of the blood group of the individual to a particular disease, the facts and figures from a very large selection of cases must be examined.

This is only feasible if the observer or observers have the necessary time to spend on the laborious testing of these cases, which must be very accurately diagnosed to be of any statistical value.

It is more feasible, however, for a multitude of observers to note their findings regarding this relation of the blood group to the cases of any diseases at their disposal or to those of a particular disease in which they are interested, and, having collected their notes, to submit them to a common group of statisticians who could file the material. When sufficient data had been collected, conclusions of probable interest and possible value might be derived from them.

For contrast as well as control purposes, cases from the two classes of diseases chosen for this article were blood-grouped. Some interesting figures were noted.

Whereas for general purposes the total percentages of blood groups of the cases of neoplasms grouped were roughly within normal limits, it was noted, on the other hand, that there was a strikingly high incidence of blood diseases in the blood groups B and AB. It was also noted that many of the severest cases of the blood diseases fell into those latter blood groups.

Several cases in which autoagglutination was present were grouped, and it was interesting to note one or two points about this phenomenon.

One would have liked to have more time to spend and a larger number of cases at one's disposal, in order that some definite ruling as to the ultimate direction of those figures could be established.

MILK, Phosphatase Test for Detection of Underpasteurized Milk, Geiger, J. C., and Davis, C. J. A. M. A. **109**: 1363, 1937.

The experience of the San Francisco Department of Public Health with the phosphatase test as advocated by Kay and Graham would indicate that adequately pasteurized milk will invariably give a negative test.

This test, therefore, offers reliable laboratory evidence to confirm proper pasteurization of milk and cream of market supplies.

The test briefly is as follows:

1. To 10 c.c. of the buffer substrate solution (one tablet containing disodium phenylphosphate and sodium barbitone is dissolved in 50 c.c. of water) contained in a 25 c.c. stoppered test tube, add 0.5 c.c. of the milk and mix thoroughly.

2. Add two drops of chloroform, stopper the tube, and incubate at from 37° to 38° C. for one hour.

3. At the end of this time, add 4.5 c.c. of the diluted Folin and Ciocalteu reagent (1 volume of reagent and 2 volumes of water), mix, allow to stand for three minutes and filter.

4. To 10 c.c. of the filtrate, add 2 c.c. of the sodium carbonate solution (14 per cent anhydrous sodium carbonate), mix thoroughly, and place in boiling water for five minutes and again filter.

5. Compare the color of the filtrate in a 13 mm. tube with the Lovibond standard 2.3 blue glass in the "limitester."

If the color of the filtrate exceeds that of the standard glass, it may be safely assumed that the milk has been improperly pasteurized; that is, the temperature has been too low or the time of heating too short.

BISMUTH, Rapid Clinical Method for Estimation of, in Urine, Hanzlik, P. J., Lehman, A. J., Richardson, A. P., and Van Winkle, W., Jr. Arch. Dermat. & Syph. 36: 725, 1937.

The method makes use of well-known principles of direct oxidation by potassium permanganate, sulfuric acid and heat, decolorization with oxalic acid to the clearness of water, and of a reducing agent to prevent oxidation of sodium iodide for reaction with bismuth in a strongly acid solution. The iodide reacts with bismuth to form sodium iodobismuthite, a colored ion which permits direct colorimetric estimation by matching with permanent

color standards on a paper scale. Only about ten minutes is required for making an estimation. The equipment is as simple as that used for other laboratory work in clinics or physicians' offices, and is readily procurable. It consists of a small iron support, a clamp, a pyrex test tube (3 by 20 cm.), and a microburner. All reagents, except concentrated sulfuric acid, are obtainable in tablet form. Undoubtedly the worst reagent to handle is concentrated sulfuric acid, but nothing has been found that will take its place. A large test tube is necessary to accommodate foaming, which is readily controlled by playing the flame of the microburner up and down the sides of the test tube. With a little practice one can easily manipulate the test tube without loss of its contents. However, a loss does not affect the accuracy of the estimation, which depends on matching concentrations.

The paper color scale was prepared by Mr. George H. Needham, of San Francisco, an expert water-color artist and microchemist, who matched standard solutions of bismuth and the same reagents (obtainable from the James H. Barry Co., 170 Van Ness Avenue, South, San Francisco). Six color standards, indicating quantities of bismuth ranging from 0.25 to 1.2 mg. per hundred cubic centimeters, are represented on the paper scale. Intermediate quantities of bismuth may be estimated by interpolation. It would be possible, of course, to use more color standards for estimating intermediate quantities with a greater range of accuracy, but this would make the scale unwieldy. A legend below the color standards gives the following information: milligrams of bismuth per hundred cubic centimeters, daily output of bismuth in 1,200 c.c. of urine (when the daily volume is known an estimation can be made accordingly), and the details of the procedure for testing urine.

The estimation of bismuth is conveniently carried out in six steps as follows:

1. Put 10 c.c. of urine into a long test tube (3 by 20 cm.).

2. Add 1 tablet (0.4 gm.) of potassium permanganate and 2 c.c. concentrated sulfuric acid. Heating will produce foaming.

3. Boil gently over a microburner for about two minutes.

4. Add 1 tablet (0.4 gm.) of oxalic acid. Decolorization will take place, and the solution should be allowed to cool.

5. Add 1 tablet (from 0.01 to 0.04 gm.) of sodium sulfite and sodium sulfate and 1 tablet (0.05 gm.) of sodium iodide. The fluid will become yellowish green if bismuth is present.

6. Match with the color scale, holding the test tube against the white margin above the standards.

If for an occasional specimen complete oxidation does not take place, indicated by the presence of some tint, the procedure from the second step on should be repeated.

The final oxidized solution must be clear as water for the proper matching of colors.

ARTHRITIS, Gonococcic, Pathogenesis, Mechanism of Recovery and Treatment, Keefer, C. S., and Spink, W. W. J. A. M. A. **109**: 1448, 1937.

A study of 140 cases of gonococcic arthritis leads to the following statements:

Gonorrhea is a frequent cause of acute polyarthritis, and, while any joint may be affected, gonococcic arthritis is most often seen in the knees, wrists, and ankles.

Associated lesions, such as tenosynovitis, bilateral metastatic catarrhal conjunctivitis, iridocyclitis, and keratodermia blennorrhagicum, are helpful clinical aids in the diagnosis.

The pathologic lesions in the joints begin in the periarticular tissues and synovial membrane, and involvement of the cartilage is secondary. Destruction of cartilage is most conspicuous in the wrist, hip, and finger joints. Bony ankylosis is rare except in the wrist joints. Fibrous ankylosis is more common.

Examination of the synovial fluid is helpful in diagnosis. Organisms were isolated in 26 per cent of the cases. The fluid had all the characteristics of an exudate. The average total cell count was higher for the infected fluid than for the sterile fluid.

Gonococcus complement fixation and bacteriolytic antibodies diffuse into the synovial cavities.

The antibody content of synovial fluid is the same or slightly less than that of the blood when the fluid is sterile. When it is infected, antibodies are not demonstrated.

The synovial fluid contains antitryptic substances which probably protect the cartilage from destruction by the tryptic-like ferments of the leucocytes.

The gonococcus complement fixation test on the blood gave a positive reaction in 86 per cent of the cases. The percentage of positive reactions was somewhat lower for tests on the synovial fluid, especially when the fluid was infected.

The mucin in synovial fluid interferes with the destruction of organisms when the antibody titer is low. When the antibody titer rises, there is no apparent depression of the activity of the fluid.

The blood plasma of patients with gonococcic infections is actively bactericidal for the homologous strain of infecting organism. The bactericidal activity increases during the course of the disease.

There is suggestive evidence that recovery from gonococcic arthritis is associated with the development of increased resistance to the invading organism.

There are various types of treatment. There is evidence that sulfanilamide is bactericidal for the gonococcus when it is added to blood serum. In two patients with infected synovial fluid the gonococci disappeared from the fluid within two days after this drug was administered. This type of treatment deserves further study and investigation.

PNEUMONIA, Pneumococcic, Community Provision for Serum Treatment of, Cecil, R. L., Bullowa, J. G. M., Chickering, H. T., and Corwin, E. H. L. J. A. M. A. **109**: 1323, 1937.

During the next few years departments of health should engage in a vigorous campaign against pneumonia. Special divisions of pneumonia service should be established under the guidance of properly qualified physicians.

Through the regular medical channels, physicians should be made cognizant of the fact that serum is lifesaving in certain types of pneumonia, and that the particular type of pneumococcic infection from which the patient may be suffering should be determined at the earliest possible moment. Free facilities for the rapid determination of the type of infection should be made available in each community at all times, day and night.

Because of the communicable nature of the pneumonias, it is highly desirable that pneumonia patients in hospitals be segregated in cubicles and that a complete aseptic technique be followed.

Pneumonia patients should be considered in the same urgent category with emergency surgical cases. Certain physicians on the attending staff should be made responsible for the treatment of these patients and should be on call day and night, as is the custom in the surgical services.

In connection with the divisions of pneumonia service of health departments, a clinical consultation service should be established to aid physicians in the administration of serum therapy and in the taking of specimens of blood and sputum for bacteriologic study.

In all instances of death from pneumonia, physicians should be requested to report the precise nature of the invading organism.

Concentrated serum for the prevailing types of pneumonia should be made available without cost to physicians requesting it, provided the type of pneumonia has been ascertained prior to the request for serum.

Control work of pneumonia and the production of therapeutic serums should not be allowed to interfere with the fundamental research activities of the laboratories.

The results obtained in New York and Massachusetts justify the appropriation of adequate funds to health departments for pneumonia control work and continued research.

HORMONES, Serologic Antibodies Against, Bauer, J. J. A. M. A. **109**: 1442, 1937.

A prolonged treatment of rabbits with injections of thyroxine leads to a resistance of these animals against thyroxine and to the appearance of serologic antibodies detectable by the complement fixation reaction.

A prolonged treatment of rabbits with diiodotyrosine, epinephrine, and frequently also insulin and phenol, is followed by the appearance of serologic antibodies detectable by the complement fixation reaction.

Most patients with hyperthyroidism give the same positive serum reaction, whereas in other individuals this reaction is negative as a rule.

The complement fixation reaction is to be obtained in almost the same way with different antigens: thyroxine, diiodotyrosine, epinephrine, sympatol, insulin, tyrosine, and phenol. Alanine has been found to give a negative reaction if used as antigen.

Three patients with spontaneous hypoglycemia gave a positive reaction, one of them with only insulin as antigen.

The extensive nonspecific complement fixation reaction involves different hormones and other substances containing a phenol group.

It is apparent that different degrees of endocrine hyperactivities, such as hyperthyroidism and hyperinsulinism as well as experimentally produced states of hyperhormonization in animals, may give rise to the appearance of nonspecific serologic antibodies.

HYPERPROTEINEMIA, Its Significance, Jeghers, H., and Selesnick, S. Internat. Clin. 3: 249, 1937.

Hyperproteinemia comprised 2.4 per cent of routine blood protein determinations made during 1936 in the clinical laboratory of one general hospital. It is found consistently enough in dehydration, multiple myeloma, lymphogranuloma inguinale, sarcoid of Boeck, leprosy, kala-azar and schistosomiasis to be of diagnostic significance. It may rarely occur in a few other diseases. In practically each instance, the increase is mainly in the globulin (more specifically the euglobulin) fraction, with an absolute diminution of albumin and a marked reversal of the albumin-globulin ratio. Even normal total protein values may be significant if the globulin shows similar increases. A few cases are on record in which Bence-Jones protein or fibrinogen constituted the protein increase. The pathologic physiology of hyperproteinemia may also be of diagnostic significance. An abnormal protein increase of the blood may cause rouleau formation of the erythrocytes, increased blood sedimentation rate, spontaneous precipitation of blood proteins, precipitation of blood proteins by Hayem's solution, discrepancy in acid-base equivalence, failure of the clot to retract, rapid coagulation of blood, intravascular autohemagglutination, and anticomplementary Wassermann reactions. Hyperproteinemia probably has little or no effect on the blood calcium level, renal function, or oncotic pressure of the blood. The Takata-Ara reaction, the formol-gel reaction, and the globulin precipitation test are usually positive in the presence of hyperglobulinemia and may be valuable simple procedures for detecting hyperproteinemia.

GONORRHEA, The Problem in the United States, Vonderlehr, R. A., and Usilton, L. J. J. A. M. A. **109**: 1425, 1937.

The cultural method is the most reliable procedure for the diagnosis of gonococcic infection, exceeding both the smear and complement fixation methods. The isolation of the gonococcus makes the diagnosis unquestionable, and a report based on this finding constitutes irrefutable evidence in court. The smear method, however, has many advantages over the cultural method, because of such features as its simplicity, inexpensiveness, and rapidity. It fails to reveal as many positive cases as the cultural method and, in some instances, false positive results are reported, owing to the inability of the examiner to differentiate the gonococcus from other bacteria. Furthermore, it is impossible to distinguish viable from nonviable gonococci.

The present methods for making complement fixation tests are unsatisfactory and frequently give false positive results.

TRANSFUSION, Kline Exclusion Test in Prevention of Syphilis, Straus, R. Arch. Dermat. & Syph. **36**: 1039, 1937.

The proper selection of a donor necessitates not only tests for compatibility of blood and physical examinations but also, immediately before the transfusion, a very sensitive blood

test for syphilis, to prevent the transmission of the disease. The Kline exclusion slide test is an excellent method for this purpose, even in emergency cases, because of the rapidity, simplicity, and ease of its performance, and its high degree of sensitivity.

BLOOD SEDIMENTATION In Pulmonary Tuberculosis, Volk, R. Am. Rev. Tubere. 36: 567, 1937.

Study of the results obtained on 1,000 sedimentation determinations shows a definite parallelism between clinical findings and sedimentation rates. Patients having clinical signs of active disease present the greatest percentage of "active curves." There was agreement to the extent of 85 to 99 per cent, which is as close a check as anyone can hope for in a nonspecific procedure.

There seems to be definite evidence that red-cell sedimentation can be a valuable adjunct to other routine procedures in the clinical study of tuberculosis. It has been found of considerable value in pneumothorax and thoracoplasty cases, where x-ray films may give us no more information than that a certain amount of collapse of the lung exists.

A repeatedly elevated sedimentation rate, with no clinical signs of activity of tuberculous disease, requires a more careful study of the x-ray films, more thorough examinations of sputa, and, if these continue to be negative, the patient should be investigated for other causes of an increased sedimentation rate.

The red-cell sedimentation should not be depended upon exclusively, because there are other factors to alter the test in individual cases, such as severe anemia, pregnancy, and intercurrent infections; but the test has shown itself of sufficient value to be included as a routine procedure in tuberculosis sanatoria.

TUBERCLE BACILLI, Comparative Study of Various Methods of Cultivation From the Blood, Galton, M. M. Am. J. Hyg. 26: 259, 1937.

From this study on the cultivation of Mycobacterium tuberculosis from blood the following conclusions may be drawn:

1. The use of untreated blood and water-hemolyzed blood sediment yielded a fair and approximately equal percentage of cultures. The acetic acid method was entirely unsatisfactory in the hands of the author.

2. With all types of the blood samples, Petragnani medium and extract blood agar were the best of the ten media employed, both with respect to the number of successful cultures and the earliest appearance of growth.

3. All egg media, except that of Petragnani, were inferior to extract blood agar.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Minor Maladies and Their Treatment*

THOSE familiar with previous editions of this book—and they must be legion for this is a book to gladden the soul of the practitioner and, most especially, the young practitioner—will recall that it deals almost entirely with the homely, everyday conditions, about which the physician is apt to be most frequently consulted.

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And so, eventually, Dr. Williams wrote this book. Those already acquainted with previous editions need only be told that this one maintains the standard set by its predecessors. Those who meet this book for the first time will recognize at once that seldom can so much practical wisdom and common sense, backed by an extensive and digested experience, be found between a single cover for so little money.

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This is a volume which should find a favorite place on the working bookshelf of the practicing physician.

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*Minor Maladies and Their Treatment. By Leonard Williams, M.D. Cloth, ed. 7, 439 pages. $3.75. William Wood & Co., Baltimore, Md.

†Muir's Bacteriological Atlas. By C. E. Van Rooyen, M.D., Harley Research Fellow and Lecturer in Bacteriology, University of Edingburgh. Cloth, ed. 2, 90 pages, 83 colored plates, $5.25. William Wood & Co., Baltimore, Md.

‡Modern Treatment in General Practice, Vol. III. Edited by Cecil P. G. Wakeley, D.Sc., Fellow of King's College, London, Senior Surgeon, King's College Hospital, Editor of "The Medical Press and Circular." Cloth, 436 pages, 54 figures. $4.00. William Wood & Co., Baltimore, Md.

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As in the previous edition, each chapter is followed by a concise summary and by a list of references.

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*The Microtomist's Vade Mecum (Balles Lee). A Handbook of the Methods and Animal and Plant Microscopic Anatomy. Edited by J. Bronte Gatenby, Professor of Zoology and Comparative Anatomy, Trinity College, Dublin, and Theophilus S. Painter, Professor of Zoology, Texas University. Cloth, ed. 10, 784 pages. $9.00. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

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While diagrammatic, they are nevertheless accurate in relationships and based upon accurate measurements and careful dissections.

Professor Grant has written a book which seems destined to take its place as a classic on this subject, and one which can be highly recommended without reserve.

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*The Therapeutic Problem in Bowel Obstructions. By Owen H. Wangensteen, M.D., Professor of Surgery, University of Minnesota. Cloth, 360 pages, 90 figures. $6.00. Charles C. Thomas, Springfield, Ill.

†A Method of Anatomy, Descriptive and Deductive. By J. C. Boileau Grant, M.C., Professor of Anatomy in the University of Toronto. Cloth, 650 pages, 564 figures. $6.00. William Wood & Co., Baltimore, Md.